US ARMY INSTITUTE OF SURGICAL RESEARCH

ANNUAL RESEARCH PROGRESS REPORT

FOR FISCAL YEAR 1988

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FORT SAM HOUSTON
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1 OCTOBER 1988

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Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to Army Regulation 70-25 and US Army Medical Research and Development Command Regulation 70-25 on the use of volunteers in research.

In conducting the research described in this report, the investigators adhered to the Animal Welfare Act and other Federal statutes and regulations relating to animals and studies involving animals and with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 86-23.

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This publication was compiled by Christine C. Davis, Research Protocol Coordinator, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012.

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SUBJECT: US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1988

The US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1988 is forwarded under the provisions of OTSG Regulation 70-31 dated 2 April 1969.

Basil A. Pruitt Jr.

BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander and Director

Encl
as

FOREWORD

> "To study the phenomena of disease without books is to sail an uncharted sea, while to study books without patients is not to go to sea at all."
>
> Sir William Osler's "Books and Men" in Aequanimitas with Other Addresses

The Institute of Surgical Research has celebrated its first 40 years as a burn center with a symposium reviewing the accomplishments of its past and present staff members and its contributions to medicine in general and military surgery in particular. In the course of providing care for severely burned patients, many of the pathogenetic factors and complications that influence morbidity and mortality have been identified. The research programs that have addressed postburn organ system dysfunction and life-threatening complications have produced information that has increased our understanding of the pathophysiology of injury and led to improvements in care that have increased survival of severely injured soldiers.

Major accomplishments selected from among the many research advances that have placed this Institute in a leadership position, both nationally and internationally, include:

1. Validation of the primacy of crystalloid fluid in burn patient resuscitation to simplify the military logistics of burn care.

2. Development of reliable techniques to diagnose inhalation injury.

3. Development of effective topical chemotherapy to prevent invasive burn wound infection.

4. Development of effective stress ulcer prophylaxis.

5. Development of prophylactic hemodialysis for patients with acute renal failure.

6. Definition of essential structural characteristics of skin substitutes.

7. Identification of the effects of burn injury and infection on leukocyte populations and functions.

8. Identification of the integrated neurohumoral responses that mediate postinjury hypermetabolism.

9. Development of nutritional regimens to prevent body wasting in burn patients.

10. Development of a computerized burn patient outcome analysis.

The 7,549 burn patients treated at this Institute in the past four decades have served as the Institute's research base in the same fashion as do patients in NIH-sponsored clinical research centers. The study of clinically evident and significant problems in burn patients ensures both the military relevance of the Institute's research and the prompt application of the research results to all injured soldiers. Patient care activities have been and remain an integral and inseparable part of the Institute's research programs. The patient base both initiates and completes the clinical problem identification, laboratory research resolution, and clinical validation feedback loop that maintains research efficiency and effectiveness and has been responsible for the noted improvements in care of the injured soldier. The Institute's patient research base provides the ship that has let the Institute sail Sir William's sea and successfully study the phenomena of burn disease.

Basil A. Pruitt Jr.

BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander and Director

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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Adults; (U) Children; (U) RAII

23. (U) The Clinical Division of this Institute is the major treatment center for thermally injured personnel of all military services as well as other eligible beneficiaries. The goals of the Division, in addition to the specialized care of severely injured patients, include the investigation of diagnostic and therapeutic technics to improve the survival and function of injured patients as well as promulgation of scientific medical information to health professionals. A literature search is performed for each protocol initiated to avoid duplication of effort.

24. (U) Thermally injured patients from the Continential United States and throughout the world are transported to this Institute for intensive, specialized treatment. Carefully controlled evaluation of new treatment technics is conducted by the professional staff.

25. (U) 8701 - 8712. Two hundred and twenty-one seriously burned patients were admitted and treated at this Institute during calendar year 1987. Current clinical research activities include host resistance studies, endocrine changes following injury, development of optimal nutritional support of the burned patient, the use of skin substitutes, and studies on the control of postinjury infection.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: Clinical Operation, Center for Treatment of Burned Soldiers

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 January 1987 - 31 December 1987

INVESTIGATORS

William F. McManus, MD, Colonel, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Donna W. Kyzar, RN, Lieutenant Colonel, AN
Nancy C. Molter, RN, Lieutenant Colonel, AN
Thomas B. Dougherty, MD, Major, MC
James C. McKay, MD, Major, MC
Anne E. Missavage, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC
Judson C. Lively, MD, Major, MD
Patricia S. Latona, RN, Major, AN
Dawn E. Carlson, RD, Major, SP
Rosendo T. Gutierrez, Major, SP
Thomas M. Summers, RN, Major, AN
Stephen H. Luster, MS, Major, SP
Theresa A. Graves, MD, Captain, MC
Mark R. Jackson, MD, Captain, MC
Frank M. Robertson, MD, Captain, MC
Leslie B. Scorza, MD, Captain, MC
Bryan S. Jordan, RN, Captain, AN
Stephen M. Pratt, MD
Debra A. Reilly, MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: Clinical Operation, Center for Treatment of Burned Soldiers

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Jan 87 through 31 Dec 87

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Two hundred and twenty-one patients were admitted to this Institute during calendar year 1987. Principal activities included care of severely burned patients, research to improve survival and function of such patients, and education and training of health care professionals and paraprofessionals. Areas of research included a study to evaluate the effectiveness and safety of Artificial Skin in the treatment of 3° flame or scald injuries, an ongoing study of 5% aqueous mafenide acetate soaks for the topical treatment of burn wounds following grafting, studies of neuroendocrine abnormalities in burn injuries, a clinical evaluation of the use of high frequency ventilation in patients with inhalation injury, quantification of dynamic splint forces on metacarpophalangeal function recovery, evaluation of _in vitro_ cultivated keratinocytes as epithelial autografts for the closure of burn wounds, evaluation of imipenem-cilastatin sodium for

prophylactic activity against bacterial pneumonias in burned patients with inhalation injury, a clinical study of the safety and efficacy of ceftazidime in the parenteral therapy of infections in hospitalized burn patients, evaluation of serum visceral protein levels as indicators of nitrogen balance, a study of medium-chain triglyceride utilization, a study of salt and water balance in the thermally injured patient, and a project to characterize certain biochemical indicators of infection in the thermally injured.

CLINICAL OPERATION
CENTER FOR TREATMENT OF BURNED SOLDIERS

During calendar year 1987, 221 patients were admitted to this Institute and there were 221 patient dispositions during the same period. Statistical data are based on the 221 patient dispositions during calendar year 1987. There were 181 males and 40 females with an average age of 28.9 yr, ranging from 3 months to 84 yr of age. The average total burn size of the entire population was 23.3% of the total body surface area, with a 15.5% average extent of full-thickness injury. The average hospital stay of all patients, excluding convalescent leave for active duty military patients, was 39.2 days. One hundred and sixty-four patients were admitted within 48 h of injury (74.2%).

During calendar year 1987, 889 operative procedures were performed on 179 patients, an average of 5 operative procedures per patient. Four hundred and sixty-three anesthetics were given to 179 patients for an average of 2.6 anesthetics per patient. One hundred and twenty-nine patients received a total of 510,708 ml of blood for an average of 3,959 ml of blood per patient.

ADMISSION DATA

The Clinical Division of this Institute admitted 221 soldiers and other authorized patients with thermal, chemical, or electric injury during calendar year 1987. Aeromedical teams from the Institute conducted 87 missions within the Continental United States to transfer 98 of the 221 patients (44.3%) admitted. Twenty-four missions were carried out by rotary-wing aircraft (27.6%) and 63 by fixed-wing aircraft (72.4%). One hundred and thirty-nine of the 221 patients (62.9%) were admitted within 24 h of injury and 164 (74.2%) were admitted within 48 h of injury. One hundred and eighty-one patients were male and 40 were female.

DISPOSITION DATA

The following statistics are based on 221 patient dispositions during calendar year 1987. The ages of these patients ranged from 3 months to 84 yr, with an average age of 28.9 yr. Burn sizes averaged 23.3% of the total body surface area, with an average full-thickness component of 15.5%. Forty-eight patients were in the pediatric age group (< 15), with an average age of 3.3 yr and an average burn size of 19.1% of the total body surface area. The average hospital stay of all dispositions was 41.8 days when convalescent leave for active duty military patients was included in the calculation and 39.2 days when convalescent leave was excluded. There were 15 patients with high voltage electric injury and 4 patients

with chemical injury. The sources of admission are identified in Table 1 and the causes of burn injury are detailed in Table 2.

TABLE 1. Sources of Admission (1987)

AREA	A	AD	AF	AFD	N/MC	ND	VAB	OTHER	TOTAL
First Army	2	0	1	0	1	0	0	0	4
Third Army	6	0	7	3	6	1	5	2	30
Fifth Army	11	10	17	7	5	0	10	90	150
Sixth Army	5	0	1	0	0	0	3	0	9
England	0	0	0	1	0	0	0	0	1
Belgium	0	0	1	0	0	0	0	0	1
Germany	3	2	1	1	5	0	0	0	12
Greece	1	0	0	0	0	0	0	0	1
Guam	0	0	0	0	1	0	0	0	1
Hawaii	0	1	0	0	0	0	0	0	1
Honduras	1	0	4	0	0	0	0	0	5
Korea	2	1	0	0	0	0	0	0	3
Panama	2	0	0	0	0	0	0	0	2
Turkey	0	0	0	1	0	0	0	0	1
TOTAL	33	14	32	13	18	1	18	92	221

A = Army, **AF** = Air Force, **N** = Navy, **M** = Marine Corps, **D** = Dependent, **VAB** = Veterans Administration Beneficiary, and **OTHER** = Civilian Emergency, US Public Health Service Beneficiary, and Bureau of Employees Compensation Beneficiary.

Two patients required hemodialysis for acute renal failure. Acute myocardial infarctions were seen in 6 patients. Inhalation injury was identified in 33 patients (14.9% based on admissions). Fifty patients (22.6%) had some associated injury (includes 33 patients with inhalation injury) which included

fractures or dislocations in 10 patients and lacerations in 6 patients.

Morbidity and Mortality. Twenty-one of the 221 dispositions (9.5%) died during calendar year 1987. Autopsies were performed in 11 (52.4%) of these hospital deaths. The average burn size of patients who died was 61.0% of the total body surface area and the full-thickness average was 43.8% of the total body surface area. Age ranged from 6 months to 84 yr. Seven of these patients (33.3%) had inhalation injury as a primary or contributing cause of death. Five patients (23.8%) had burn injuries exceeding 80% of the total body surface area. Four patients died with acute myocardial infarctions. Four of the 21 deaths (19.0%) occurred in pediatric patients. These children had an average total body surface area burn of 54.9% and an average full-thickness burn of 41.4%. The average age of children who died was 18 months (6-30 months). One of these children had an autopsy.

Infection was once again the most common complication following thermal injury, with 40 bacterial pneumonias occurring in 34 patients. The most common organisms isolated in patients with bacterial pneumonia were *Staphyloccocus aureus* in 27 patients, *Klebsiella* species in 10 patients, and *Escherichia coli* in 6 patients. However, only 5 patients demonstrated septicemia and 1 patient had bacterial invasion of the burn wound.

Table 3 lists the effect of age and extent of inju survival and Table 4 lists mortality rates associated w increments of 10% of the total body surface area for the years 1984-87. Table 5 summarizes the survival of patients with extensive burns from 1963-87. Table 6 compares mortality before and after the use of topical chemotherapy on the burn wound. Table 7 lists the causes of death for calendar year 1987.

EDUCATIONAL ACTIVITIES

During calendar year 1987, the professional staff of the Clinical Division continued to provide education to professional and paraprofessional groups at the local, national, and international levels. A total of 28 resident physicians were attached for periods of 1-3 months, including 8 from Wilford Hall USAF Medical Center, 5 from Letterman Army Medical Center, four each from William Beaumont Hospital (Royal Oak MI) and Pensacola Naval Air Station, two from Providence Hospital (Southfield MI), and 1 each from Brooke Army Medical Center, Fitzsimons Army Medical Center, William Beaumont Army Medical Center, Travis Air Force Base Medical Center, and the University of Texas Health Science Center (San Antonio TX). Six interns from Brooke Army Medical Center (San Antonio TX) rotated through the Institute. A total of 8 medical students

TABLE 2. Burn Etiology (1987)

Causes	Number of Patients	Disposition (%)	Deaths	Mortality (%)
Hot Liquids	52	23.5	2	3.8
Gasoline, Diesel, and Kerosene	50	22.6	3	6.0
Structural Fires	24	10.9	7	29.2
Open Flames	23	10.4	5	21.7
Electrical	17	7.7	-	-
Butane, Propane, or Natural/Sewer Gas Explosions	16	7.2	4	25.0
Motor Vehicle Accidents	12	5.4	-	-
Aircraft Accidents	9	4.1	-	-
Bomb, Shell, Simulator Grenade, and Gunpowder Explosions	5	2.3	-	-
Chemical	4	1.8	-	-
Contact	4	1.8	-	-
Smoking, Clothes Ignition	4	1.8	-	-
Welding	1	0.5	-	-
TOTAL	221	100.0	21	9.5

TABLE 3. Age, Body Surface Involvement, and Mortality (1987)

Age (Years)	Total Body Surface Area Burn (%) 0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total Cases	Total Deaths	Mortality (%)
0 - 1	1	5	-	-	1	1	-	-	-	-	8	1	12.5
1 - 2	3	5	2	-	1	1	1	-	-	-	13	1	7.7
2 - 3	2	5	1	-	1	-	-	-	-	-	9	1	11.1
3 - 4	0	2	1	-	1	-	-	-	-	-	4	-	-
4 - 5	1	1	-	-	-	-	-	-	-	-	2	-	-
5 - 10	4	1	1	1	-	-	-	-	-	-	7	-	-
10 - 15	1	-	1	1	1	-	-	-	-	-	4	-	-
15 - 20	4	6	1	1	1	-	-	-	-	-	13	1	7.7
20 - 30	28	14	7	6	7	5	2	-	-	-	69	1	1.4
30 - 40	11	6	5	6	3	3	1	-	-	1	36	2	5.6
40 - 50	5	3	4	2	2	1	2	-	-	1	20	1	5.0
50 - 60	3	3	7	1	-	1	1	-	-	-	16	3	18.8
60 - 70	2	1	5	1	-	-	2	-	2	1	14	5	35.7
70 - 80	2	-	1	-	-	-	1	-	-	-	4	2	50.0
80 - 90	-	-	-	1	1	-	-	-	-	-	2	2	100.0
90 - 100	-	-	-	-	-	-	-	-	-	-	-	-	-
Total Cases	67	52	36	20	19	12	10	-	2	3	221		
Total Deaths	-	-	1	2	3	4	6	-	2	3		21	
Mortality (%)	-	-	2.8	10.0	15.8	33.3	60.0	-	100.0	100.0			9.5

TABLE 4. Total Body Surface Burn Involvement (%) and Mortality (1984-7)

	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
1987											
Number of Patients	67	52	36	20	19	12	10	-	2	3	221
Number of Deaths	-	-	1	2	3	4	6	-	2	3	21
MORTALITY (%)	-	-	2.8	10.0	15.8	33.3	60.0	-	100.0	100.0	9.5
1986											
Number of Patients	61	40	32	21	19	7	11	7	4	5	207
Number of Deaths	1	2	2	2	5	2	4	2	4	5	29
MORTALITY (%)	1.6	5.0	6.3	9.5	26.3	28.6	36.4	28.6	100.0	100.0	14.0
1985											
Number of Patients	41	46	28	28	19	11	9	6	5	4	197
Number of Deaths	2	3	5	3	7	3	5	5	5	4	42
MORTALITY (%)	4.9	6.5	17.9	10.7	36.8	27.3	55.6	83.3	100.0	100.0	21.3
1984											
Number of Patients	46	38	31	23	18	13	7	5	6	3	190
Number of Deaths	-	-	2	8	4	6	2	3	6	3	34
MORTALITY (%)	-	-	6.5	34.8	22.2	46.2	28.6	60.0	100.0	100.0	17.9

TABLE 5. Survival and Nonsurvival by Year for Patients with Burns > 30% of the Total Body Surface Area (1963-87)

Year	SURVIVORS Number of Cases	SURVIVORS Average Burn (%) Total	SURVIVORS Average Burn (%) 3°	NONSURVIVORS Number of Cases	NONSURVIVORS Average Burn (%) Total	NONSURVIVORS Average Burn (%) 3°
1987	46	43.7	17.2	21	63.0	44.9
1986	178	21.8	7.3	29	59.8	41.4
1985	48	43.6	21.7	42	54.3	37.1
1984	43	46.4	24.8	32	59.5	38.7
1983	37	43.5	17.5	30	62.8	50.7
1982	53	43.7	24.8	54	53.9	38.3
1981	54	42.7	17.5	43	62.2	39.8
1980	62	42.7	15.1	66	64.3	41.8
1979	61	45.4	13.4	74	65.0	37.0
1978	67	45.7	14.8	69	55.2	33.0
1977	66	42.2	14.4	70	56.9	29.0
1976	69	45.5	15.0	79	64.2	31.1
1975	80	46.1	14.7	94	61.3	32.8
1974	55	43.9	12.2	97	60.8	35.9
1973	47	43.7	19.6	113	60.3	36.2
1972	62	42.0	17.2	103	56.7	35.9
1971	63	41.9	14.0	68	60.8	38.0
1970	92	39.4	10.7	70	51.9	32.6
1969	113	43.2	11.1	70	58.7	26.4
1968	143	44.2	12.6	38	54.6	24.6
1967	103	42.7	13.3	51	59.9	32.3
1966	68	41.5	14.9	59	59.9	31.3
1965	47	43.8	21.0	33	66.0	33.4
1964	40	41.8	14.8	37	65.0	42.4
1963	28	45.8	19.6	57	69.0	41.0

TABLE 6. Comparison of Burn Mortality Rates (1962-3 and 1964-87)

	TOTAL BODY SURFACE AREA BURN (%)														
	0-30			30-40			40-50			50-60			60-100		
YEARS	Number of Patients	Number of Deaths	Mortality (%)	Number of Patients	Number of Deaths	Mortality (%)	Number of Patients	Number of Deaths	Mortality (%)	Number of Patients	Number of Deaths	Mortality (%)	Number of Patients	Number of Deaths	Mortality (%)
1962-63	140	6	4.3	36	16	44.4	35	22	61.1	23	18	78.3	55	49	89.1
1964-86	3,020	114	3.8	837	155	18.5	667	212	31.8	474	227	47.9	870	725	83.3
1987	155	6	3.9	20	2	10.0	19	1	5.3	12	3	25.0	15	9	60.0

TABLE 7. Causes of Death (1987)

Patient	Age	Sex	BURN (%) Total	BURN (%) 3	Postburn Day	Cause of Death
1	82	M	46	28	26	*46% total body surface area burn with bronchopneumonia.
2	55	M	50	15	26	*50% total body surface area burn with inhalation injury and bronchopneumonia.
3	63	F	90	64	16	*90% total body surface area burn with inhalation injury and bronchopneumonia.
4	1	F	60	38	33	*60% total body surface area burn with bronchopneumonia.
5	70	M	65	65	1	*65% total body surface area burn with acute myocardial failure and DIC.
6	0	M	58	27	22	*58% total body surface area burn with Candida burn wound sepsis.
7	50	F	69	57	9	*69% total body surface area burn with bronchopneumonia.
8	63	M	68	26	19	68% total body surface area burn with acute myocardial infarction and bronchopneumonia.
9	38	M	42	11	6	*42% total body surface area burn with aspiration of gastric contents.

*Autopsy not performed.

TABLE 7 (Continued)

Patient	Age	Sex	BURN (%) Total	3	Postburn Day	Cause of Death
10	65	F	70	28	49	*70% total body surface area burn with diabetes mellitus and fungal burn wound sepsis.
11	84	M	31	10	2	31% total body surface area burn with acute myocardial infarction.
12	77	F	21	21	30	21% total body surface area burn with acute myocardial infarction.
13	2	M	43	43	8	*43% total body surface area burn with inhalation injury.
14	37	M	94	92	21	94% total body surface area burn with bronchopneumonia.
15	1	F	59	59	16	59% total body surface area burn with inhalation injury and pneumonia.
16	49	M	64	64	15	64% total body surface area burn with inhalation injury.
17	26	M	58	58	15	58% total body surface area burn with inhalation injury and bronchopneumonia.
18	60	M	85	18	13	85% total body surface area burn with acute adrenal infarctions.
19	47	M	91	86	33	91% total body surface area burn with fungal burn wound infection.

*Autopsy not performed.

TABLE 7 (Continued)

Patient	Age	Sex	BURN (%) Total	BURN (%) 3	Postburn Day	Cause of Death
20	59	M	36	30	22	36% total body surface area burn with inhalation injury and pneumonia.
21	69	M	84	83	14	84% total body surface area burn with pneumonia and DIC.

*Autopsy not performed.

rotated through the Institute, including 5 students from the Indiana University School of Medicine and 1 each from the Uniformed Services University of Health Sciences, Tufts University, and the University of Oregon Medical School. A total of 22 physicians visited from foreign countries for periods ranging from 1 day to 1 yr, which included 4 each from Guatemala and Pakistan, 3 each from Japan and Norway, 2 from Burma, and 1 each from Malaysia, France, Israel, the United Kingdom, Canada, and Egypt. The Respiratory Therapy Branch had 152 trainees, the Physical Therapy Branch had 99 trainees, and the Occupational Therapy Branch had 103 trainees. Twenty-three scientific publications appeared in refereed medical journals and approximately 209 scientific presentations were conducted for military and civilian audiences. Numerous scientific presentations were made at the Academy of Health Sciences and various military installations throughout the continential United States, to include support of the Combat Casualty Care Courses for the United States Army. In addition, weekly professional staff conferences were conducted for and by Institute personnel.

PRESENTATIONS

Latona PS: Initial management of burn-injured victims. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 12 January 1987.

Summers TM: Communicating effectively. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 12 January 1987.

Hollan E: Infection control and the burn patient. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 12 January 1987.

Lively JC: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 13 January 1987.

Jordan BS: Cardiac output. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 January 1987.

Jordan BS: Cardiac output. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 January 1987.

Burleson DG: Anti-LeuM3 as an adjunct to accurately identify minor lymphocyte subpopulations in burned patients. Presented at the Conference on Immune Consequences of Traumatic Injuries, Snowbird, Utah, 20 January 1987.

Jordan BS: High frequency ventilation and the burn patient. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 20-21 January 1987.

Shippee RL: The effect of burn injury and zinc nutriture on peripheral blood T lymphocyte subpopulation distribution in a murine model. Presented at the Immune Consequences of Thermal and Traumatic Injury Symposium, Snowbird, Utah, 21 January 1987.

Gutierrez RT: Closing address. Presented to the Physical Therapy Specialist Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 22 January 1987.

Carlson DE: Combat field feeding system - force development and experimentation. Presented to Dietetic Interns, Nutrition Care Division, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 23 January 1987.

Summers TM: Psychosocial aspects of burn care. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 January 1987.

Wallace A: Burn wound management. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 January 1987.

Wallace A: Stop, look, and listen. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 January 1987.

Pruitt BA Jr: Early excision of burns. Presented at the 25th Anniversary Symposium, Parkland Memorial Hospital Burn Center, Dallas, Texas, 26-27 January 1987.

Cioffi WG Jr: Inhalation injury and pneumonia. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Graves TA: Burn wound management: topical agents, biological dressings, synthetics, and excision and grafting. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Gutierrez RT: Overview of the US Army Medical Research and Development Command and the US Army Institute of Surgical Research. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences,

Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Gutierrez RT: Panel Discussion: Biomechanical complications. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Gutierrez RT: Panel Discussion: Rehabilitation - occupational and physical therapy. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Gutierrez RT: Physical therapy in burn care. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Jordan BS: Functioning in an ICU environment. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Jordan BS: Management of pain. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Jordan BS: Review of current research at the US Army Institute of Surgical Research. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Kyzar DW: Panel Discussion: Experiences of nonphysician medical staff in the combat zone (Vietnam) and in El Salvador. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Latona PS: Initial care of the burn patient. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Luster SH: Occupational therapy in burn care. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Luster SH: Panel Discussion: Biomechanical complications. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Luster SH: Panel Discussion: Rehabilitation - occupational and physical therapy. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Mason AD Jr: Perspectives in clinical research. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Missavage AE: Management of infection, sepsis, and suppurative thrombophlebitis. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Pratt SM: Cardiovascular, gastrointestinal, and renal complications. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Pruitt BA Jr: Pathophysiology of thermal injuries: Triage and initial care. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Reilly DA: Nutritional care. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Robertson FM: Fluid resuscitation, escharotomy, and fasciotomy. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Summers TM: Psychological complications. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Zellers LA: Air transport of burn patients. Presented at the OT/PT Conference on Management of Burns in the Theater of

Operations, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 January-6 February 1987.

Latona PS: Initial management of the burn patient. Presented at Saint Rose Hospital, San Antonio, Texas, 27 January 1987.

McManus WF: Resuscitation of the burn patient. Presented at the 25th Anniversary Symposium, Parkland Memorial Hospital Burn Center, University of Texas Health Science Center, Dallas, Texas, 27 January 1987.

Summers TM: Communicating effectively. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 28 January 1987.

Pruitt BA Jr: Treatment of the cutaneous injury. Presented at the Vesicant Workshop, Aberdeen Proving Grounds, Maryland, 3-5 February 1987.

Pruitt BA Jr: Recent advances in burn care. Presented to the American College of Veterinary Surgeons, San Antonio, Texas, 5 February 1987.

Jordan BS: Wound care and later complications. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1987.

Latona PS: Initial management of burns. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1987.

Latona PS: Initial management of the burn victim. Presented to the 328th General, Fort Sam Houston, San Antonio, Texas, 5 February 1987.

Latona PS: Pediatric burn patients: Are they different? Presented at the 57th Air Evacuation Squadron Burn Symposium, Scott Air Force Base, Belleville, Illinois, 10 February 1987.

Latona PS: Wound care and later complications. Presented at the 57th Air Evacuation Squadron Burn Symposium, Scott Air Force Base, Belleville, Illinois, 10 February 1987.

Summers TM: Psychosocial aspects of burn care. Presented at the 57th Air Evacuation Squadron Burn Symposium, Scott Air Force Base, Belleville, Illinois, 10 February 1987.

Miller T: Initial management of the burn patient. Presented to the Intensive Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 12 February 1987.

Vaughan GM: Burns and nonthyroidal illness. Presented at the Endocrine Clinical Conference, Division of Endocrinology, Department of Medicine. University of Texas Health Science Center, San Antonio, Texas, 12 February 1987.

Latona PS: Initial management of the burn patient. Presented at Wesleyan College, Macon, Georgia, 13 February 1987.

McManus WF: Stress ulcer disease in the burned patient. Presented at the New Concepts in Critical Care Symposium, Tufts University School of Medicine, Sarasota, Florida, 27 February 1987.

Pruitt BA Jr: Recent advances in burn care. Presented to the American College of Veterinary Surgeons, San Antonio, Texas, 27 February 1987.

Gutierrez RT: Physical therapy in burn care. Presented to the Physical Therapy Specialist Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 2 March 1987.

Jordan BS: Wound management. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 March 1987.

Latona PS: Initial management of burns. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 March 1987.

Sandoval CM: Perioperative care of the burn patient. Presented to the Operating Room Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 10 March 1987.

Wallace A: Stop, look, and listen. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 March 1987.

McManus WF: Innovations in burn care. Presented to the Department of Medicine, Brackenridge Hospital, Austin, Texas, 12 March 1987.

Carlson D: Nutrition and wound healing. Presented to the Advanced Physical Therapy Course, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 17 March 1987.

Gutierrez RT: Initial treatment - debridement. Presented to the Advanced Physical Therapy Course, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 17 March 1987.

Gutierrez RT: Physical therapy in burn care. Presented to the Advanced Physical Therapy Course, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 17 March 1987.

Gutierrez RT: Range of motion and splinting of burns. Presented to the Advanced Physical Therapy Course, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 17 March 1987.

McManus WF: Medical readiness - thermal trauma under battlefield conditions. Presented to the Advanced Physical Therapy Course, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 17 March 1987.

Vaughan GM: Endocrinology Board Review: Diabetes insipidus and SIADH. Presented to the Division of Endocrinology, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, 19 March 1987.

Pruitt BA Jr: Current management of burn patients. Presented to the Department of Surgery, Morristown Memorial Hospital, Morristown, New Jersey, 25 March 1987.

Allen RC: Immunologic effects of burn injury. Presented to the Buffalo Surgical Society, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 28 March 1987.

Cioffi WG Jr: Inhalation injury and pulmonary complications. Presented to the Buffalo Surgical Society, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 28 March 1987.

McManus AT: Infection surveillance in burn patients. Presented to the Buffalo Surgical Society, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 28 March 1987.

McManus WF: Resuscitation of burn patients. Presented to the Buffalo Surgical Society, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 28 March 1987.

Missavage AE: Burn wound excision and closure of the burn wound. Presented to the Buffalo Surgical Society, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 28 March 1987.

Pruitt BA Jr: Epidemiology and triage of burn patients. Presented to the Buffalo Surgical Society, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 28 March 1987.

Pruitt BA Jr: Overview of techniques of burn care. Presented to the Buffalo Surgical Society, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 28 March 1987.

Vaughan GM: Neuroendocrine effects of burn injury. Presented to the Buffalo Surgical Society, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 28 March 1987.

Hollan E: Infection control in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 30 March 1987.

Shippee RL: Effect of burn injury on zinc excretion and tissue zinc distribution. Presented at the 71st Annual Meeting of the Federation of American Societies for Experimental Biology, Washington, DC, 30 March 1987.

Latona PS: Initial management of burns. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 1 April 1987.

Summers TM: Psychosocial aspects of burn care. Presented to the Chaplains, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 6 April 1987.

Pruitt BA Jr: Surgical research and the military. Presented at the Gary P. Wratten Surgical Symposium, William Beaumont Army Medical Center, El Paso, Texas, 8 April 1987.

Luster SH: Occupational therapy in burn care. Presented at the Annual Meeting of the Insurance Adjustors of America, San Antonio, Texas, 8 April 1987.

Pruitt BA Jr: Infection and burn care in the combat zone. Presented to the Combat Casualty Management Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 9 April 1987.

Pruitt BA Jr: Current treatment of the burn wound. Presented to the Throckmorton Surgical Society, Des Moines, Iowa, 10 April 1987.

Pruitt BA Jr: Fluid resuscitation in burns. Presented to the Throckmorton Surgical Society, Des Moines, Iowa, 10 April 1987.

Cavazos JS: Management of the burn victim. Presented at Incarnate Word College, San Antonio, Texas, 10 April 1987.

Mozingo DW: Acalculous cholecystitis diagnosed by indium-III leukocyte scan in a severely burned patient.

Exhibited at the Southwestern Surgical Congress, San Diego, California, 23 April 1987.

Wallace A: Nursing care of burn patients. Presented to the Recruiting Battalion, Santa Anna, California, 24 April 1987.

Zelenka JP: The role of occupational therapy in the care of burn patients. Presented to the Occupational Therapy Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 24 April 1987.

Kyzar DW: Overview of the US Army Institute of Surgical Research. Presented to the Recruiting Command Nurse Educator Tour, Fort Sam Houston, San Antonio, Texas, 28 April 1987.

McManus WF: Sulfamylon®. Presented at the Symposium on Wound Healing, 19th Annual Meeting of the American Burn Association, Washington, DC, 29 April 1987.

Pruitt BA Jr: Pathology of the burn wound. Presented at the 19th Annual Meeting of the American Burn Association's Symposium on Wound Healing, Washington, DC, 29 April 1987.

McManus AT: Relationship of sulfonamide sensitivity to in vitro activity of silver-sulfadizine. Presented at the 19th Annual Meeting of the American Burn Association, Washington, DC, 30 April 1987.

Kim SH: Mycotic disease in burn patient autopsies. Presented at the 19th Annual Meeting of the American Burn Association, Washington, DC, 1 May 1987.

McManus AT: A comparison of quantitative microbiology and histopathology in divided burn wound biopsies. Presented at the 19th Annual Meeting of the American Burn Association, Washington, DC, 1 May 1987.

Miller TM: Air-vented mattresses within the critical burn care area: how clean are they? Presented at the 19th Annual Meeting of the American Burn Association, Washington, DC, 1 May 1987.

Pruitt BA Jr: Panel Discussion: Ethical concerns. Presented at the 19th Annual Meeting of the American Burn Association, Washington, DC, 1 May 1987.

Chu C-S: Salvage of experimental full-thickness scalds with cooling and weak anodal direct current. Presented at the 19th Annual Meeting of the American Burn Association, Washington, DC, 2 May 1987.

Shimazu TS: Effects of PEEP on ventilation-perfusion ratios following smoke inhalation injury in a sheep model. Presented at the 19th Annual Meeting of the American Burn Association, Washington, DC, 2 May 1987.

Pruitt BA Jr: Combat care of burn patients. Presented to the USAFR WARMED Group, San Antonio, Texas, 5 May 1987.

Pruitt BA Jr: Infection and burn problems. Presented to the Physician's Assistant Course (C4A), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 7 May 1987.

Gutierrez RT: Physical management of burns. Presented as part of the US Army-Baylor University Program, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 8 May 1987.

Latona PS: Initial management of the burn victim. Presented as part of the US Army-Baylor University Program, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 8 May 1987.

Pruitt BA Jr: The immunologic consequences of severe injury. Presented to the Colorado Chapter of the American College of Surgeons, Denver, Colorado, 15-16 May 1987.

Pruitt BA Jr: Current activities of the American Board of Surgery. Presented to the Colorado Chapter of the American College of Surgeons, Denver, Colorado, 15-16 May 1987.

Wallace A: Physiology of the burn wound. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 18 May 1987.

Summers TM: Communicating effectively. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 18 May 1987.

Wallace A: Stop, look, and listen. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 19 May 1987.

Jordan BS: Wound management and complications of the burn wound. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 19 May 1987.

McManus WF: Infections in surgical patients. Presented to the Family Practice Association, McAllen, Texas, 19 May 1987.

Summers TM: Observation/documentation of psychosocial needs. Presented to the Nursing Service Branch, US Army

Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 19 May 1987.

Gutierrez RT: Physical therapy in burn care. Presented to Family Support Group Members, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 May 1987.

Pruitt BA Jr: Necrotizing soft tissue infections. Presented at the 14th Annual Warren H. Cole Symposium, University of Illinois College of Medicine, Chicago, Illinois, 28 May 1987.

Pruitt BA Jr: Treatment and prevention of infection in the immunocompromised surgical patient. Presented at the 14th Annual Warren H. Cole Symposium, University of Illinois College of Medicine, Chicago, Illinois, 28 May 1987.

Latona PS: Initial management of the burn victim. Presented at the Surviving Terrorism Symposium, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 29 May 1987.

Reilly DA: Intraabdominal infection in the burn patient. Presented to the International Congress on Intraabdominal Infections, Hamburg, Federal Republic of Germany, 3 June 1987.

Pruitt BA Jr: Panel Discussion: Intensive care - multi system organ failure. Presented to the International Congress on Intraabdominal Infections, Hamburg, Federal Republic of Germany, 3 June 1987.

Gutierrez RT: Mobilization to El Salvador. Presented to the Clinical Faculties Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 3 June 1987.

Latona PS: Initial management of the burn victim. Presented at the Nursing Medical Readiness Symposium, Kessler Air Force Base, Biloxi, Mississippi, 3 June 1987.

Reilly DA: Intraabdominal infection in the burned patient. Presented to the International Congress on Intraabdominal Infections, Hamburg, Federal Republic of Germany, 3 June 1987.

Summers TM: Psychosocial aspects of the burn victim. Presented at the Nursing Medical Readiness Symposium, Kessler Air Force Base, Biloxi, Mississippi, 3 June 1987.

Gutierrez RT: Physical therapy management of burns. Presented to the Physical Therapy Specialist Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 June 1987.

Latona PS: Initial management of the burn victim. Presented to the 32nd Air Medical Squardon, Kelly Air Force Base, San Antonio, Texas, 7 June 1987.

Carlson DE: The role of the dietitian in the critical care setting: Critical knowledge for dietitians. Presented to the Nutritional Assessment Postgraduate Short Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 8 June 1987.

Vaughan GM: Thyrotrophin and the thyroid axis in burn patients: Different patterns of alteration in survivors and nonsurvivors. Presented at the 69th Annual Meeting of the Endrocrine Society, Indianapolis, Indiana, 10 June 1987.

Jordan BS: Wound care and later complications. Presented to the Critical Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 June 1987.

Latona PS: Initial management of the burn patient. Presented to the Critical Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 June 1987.

Dimmick DJ: The role of the LVN in the care of the burn patient. Presented at the Madisonville State Vocational Technical School, Madisonville, Kentucky, 15 June 1987.

Gutierrez RT: Physiological response to the burn injury and care of minor burns. Presented to the Physical Therapy Specialist Advanced Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 June 1987.

Wallace A: Initial care of the burn patient. Presented to the American Association of Critical Care Nurses, San Antonio, Texas, 17 June 1987.

Gutierrez RT: Getting mobilized to El Salvador, Central America. Presented to the Physical Therapy Specialist Advanced Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 19 June 1987.

Carlson DE: Nutritional management of the burn patient. Presented to the Nutritional Assessment Postgraduate Short Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 19 June 1987.

Carlson DE: Panel Discussion: Use of the clinical nutrition specialist ASI. Presented to the Nutritional Assessment Postgraduate Short Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 26 June 1987.

Pruitt BA Jr: What's new in burns. Presented at the International Society for Burn Injuries Research Symposium, Geneva, Switzerland, 26 June 1987.

Pruitt BA Jr: Now you see it, now you don't. Presented at the International Society for Burn Injuries Research Symposium, Geneva, Switzerland, 26 June 1987.

Pruitt BA Jr: Management of patients with extensive burns. Presented at the German Army Burn Unit, Koblenz, West Germany, 27 June 1987.

Hollan E: Infection control in the AIDS era. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 1 July 1987.

Summers TM: Introduction to the hospital ministry. Presented to the Chaplain Candidate Hospital Ministry Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 6 July 1987.

Hollan E: Infection control and the burn patient. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 July 1987.

Jordan BS: Burn wound management. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 July 1987.

Latona PS: Initial management of the burn patient. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 July 1987.

Latona PS: Physiology of the burn wound. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 July 1987.

Summers TM: Communicating effectively. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 13 July 1987.

Gutierrez RT: Physical therapy in burn care. Presented to Family Support Group Members, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 14 July 1987.

Latona PS: Stop, look, and listen. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 14 July 1987.

Summers TM: Observation/documentation of psychosocial needs. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 14 July 1987.

Summers TM: Psychosocial aspects of burn care. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 14 July 1987.

Sandoval CM: Perioperative care of the burn patient. Presented to the Operating Room Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 21 July 1987.

Pruitt BA Jr: Current treatment of the burn wound. Presented at the Martinez Veterans Administration Medical Center, Martinez, California, 22-23 July 1987.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in man. Presented at the Martinez Veterans Administration Medical Center, Martinez, California, 22-23 July 1987.

Latona PS: Initial management of the burn patient. Presented at Laughlin Air Force Base, Del Rio, Texas, 31 July 1987.

Luster SH: Upper extremity evaluation and treatment course. Presented to Occupational Therapy Officers, Fort Knox, Kentucky, 5-15 August 1987.

Vaughan GM: Sophomore endocrine pathophysiology: case studies - disorders of the pituitary. Presented to the Division of Endocrinology, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, 10 August 1987.

Luster SH: Predicting daily occupational staff requirements in a burn center. Presented to the Mary Lipscomb Hamrick Army Medical Specialist Corps Research Course, Xerox International Training Center, Leesburg, Virginia, 13 August 1987.

Vaughan GM: Sophomore endocrine pathophysiology: Case studies - diabetes. Presented to the Division of Endocrinology, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, 17 August 1987.

Gutierrez RT: Current trends in burn research and physical therapy. Presented to Physical Therapy Staff, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 18 August 1987.

Latona PS: Nursing care of the burn patient. Presented at the Baptist Memorial Hospital School of Nursing, San Antonio, Texas, 31 August 1987.

Latona PS: Nursing care of the burn patient in the emergency room. Presented to the Emergency Nursing Care Course, Fitzsimons Army Medical Center, Aurora, Colorado, 2 September 1987.

McManus AT: Pseudomonas aeruginosa a waning burn pathogen? A 10 year review of 2216 admissions. Presented at the 1st International Conference of the Hospital Infection Society, London, England, 2 September 1987.

Latona PS: Initial management of the burn patient. Presented at Baptist Memorial Hospital School of Nursing, San Antonio, Texas, 4 September 1987.

Keenan JR: Initial management of the burn patient. Presented at the Preceptee Orientation, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 10 September 1987.

Luster SH: Occupational therapy in burn care. Presented at the Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 10 September 1987.

Gutierrez RT: Physical therapy in burn care. Presented to Brackenridge Hospital Physical Therapists, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1987.

Gutierrez RT: Physical therapy in burn care. Presented to the Wilford Hall USAF Medical Center Physical Therapy Officers' Advanced Course, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1987.

Pruitt BA Jr: Panel Discussion: Current concepts in surgical infection. Presented at the 32nd World Congress of Surgery, Sydney, Australia, 23 September 1987.

Pratt SM: Infections in burns: The unusual unsuspected sites. Presented at the 32nd World Congress of Surgery, Sydney, Australia, 25 September 1987.

Pruitt BA Jr: Panel Discussion: What's new in burn care. Presented at the 32nd World Congress of Surgery, Sydney, Australia, 25 September 1987.

Pruitt BA Jr: Treatment of patients with massive burns and predictors of outcome. Presented at the 12th Annual Scientific

Meeting of the Australian and New Zealand Burn Association, Adelaide, Australia, 28 September 1987.

Gutierrez RT: Physical therapy in burn care. Presented to Family Support Group Members, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 29 September 1987.

Carlson DE: Nutrition in thermal injury. Presented to the Hospital Food Service Specialist Advanced Course (800-94F30), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 30 September 1987.

Pruitt BA Jr: Surgical treatment of patients with massive burns. Presented at the Royal Children's Hospital, Melbourne, Australia, 30 September 1987.

McManus WF: Management of the massive burn. Presented to the Department of Surgery, Cornell Medical Center, New York, New York, 1 October 1987.

Carlson DE: Combat field feeding system force development test and experimentation. Presented to Dietetic Interns, Nutrition Care Division, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 8 October 1987.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 15 October 1987.

Burleson DG: Measurement of peripheral blood lymphocyte subpopulations in burned patients. Presented at the 11th International RES Congress and 24th National RES Meeting, Kauai, Hawaii, 17 October 1987.

Hollan E: Infection control in the AIDS era. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 21 October 1987.

Pruitt BA Jr: Overview of the activities of the US Army Institute of Surgical Research. Presented at the BioVision '87 Conference, San Antonio, Texas, 22 October 1987.

Vaughan GM: Control of TSH after burn injury. Presented at the 4th Annual Meeting of the US Army Regional Meeting of the American College of Physicians, San Francisco, California, 25 October 1987.

Chu C-S: Effects of low voltage direct current on the burn wound. Presented at the 40th Anniversary Symposium at the US

Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

McManus WF: Management of patients with massive burns. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

Pruitt BA Jr: Four decades of progress in burn care and research. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

Shimazu T: Effects of smoke inhalation injury on ventilation-perfusion ratio of the lung. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

Burleson DG: Effect of burn injury and infection on lymphocyte populations. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

Kim SH: Histologic classification of burn wound infections. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 October 1987.

McManus AT: The rise and fall of Pseudomonas. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 October 1987.

Vaughan GM: Central nervous system alterations in flow-phase burn injury. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 October 1987.

Gutierrez RT: PT/OT in the management of burns. Presented to Physical Therapy Assistant, Occupational Therapy, and Licensed Vocational Nurse students at St. Phillips College, San Antonio, Texas, 28 October 1987.

McManus WF: Thermal injury. Presented to the Federal Drug Enforcement Agency, San Antonio, Texas, 29 October 1987.

Luster SH: Occupational therapy rehabilitation in burn care. Presented to Physical Therapy Assistant, Occupational Therapy, and Licensed Vocational Nurse students, St. Phillips College, San Antonio, Texas, 29 October 1987.

Zelenka JP: New strategies in occupational therapy in burn care. Presented at the Annual Great Southern Occupational Therapy Conference, New Orleans, Louisiana, 29 October 1987.

Kim SH: Clinical sepsis and burn wound biopsy. Presented at the 81st Annual Scientific Assembly of the Southern Medical Association, San Antonio, Texas, 1 November 1987.

Pruitt BA Jr: Current techniques of burn wound care. Presented at the 81st Annual Scientific Assembly of the Southern Medical Association, San Antonio, Texas, 2 November 1987.

Duncan DJ: Pediatric burn patients: Are they different? Presented to the Northeast Regional Recruiting Command, St. Mary's College, Newburgh, New York, 2-3 November 1987.

Pruitt BA Jr: Use of antimicrobials in burn wound care. Presented at the 81st Annual Scientific Assembly of the Southern Medical Association, San Antonio, Texas, 3 November 1987.

Pruitt BA Jr: Effect of injury on host defenses. Presented at the Department of Surgery Trauma Meeting, Wayne State University, Detroit, Michigan, 5-7 November 1987.

Pruitt BA Jr: Nonbacterial infections in burn patients. Presented at the Department of Surgery Trauma Meeting, Wayne State University, Detroit, Michigan, 5-7 November 1987.

Keenan JR: Initial management of the burn patient. Presented at St. Phillips College, San Antonio, Texas, 10 November 1987.

McManus WF: Burn disaster management. Presented at the 94th Annual Meeting of the Association of Military Surgeons of the United States, Las Vegas, Nevada, 10 November 1987.

Pruitt BA Jr: Burn wound care and treatment for chemical burns, burn patient transfer and transport. Presented to the Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 10 November 1987.

Pruitt BA Jr: Principles of resuscitation and diagnosis and treatment of inhalation injury. Presented to the Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 10 November 1987.

Gutierrez RT: Physical therapy in natural disasters. Presented at the 94th Annual Meeting of the Association of Military Surgeons of the United States, Las Vegas, Nevada, 10 November 1987.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 19 November 1987.

Hollan E: Infection control in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 1 December 1987.

Gutierrez RT: Physical therapy in burn care. Presented to the Physical Therapy Advanced Course, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 1 December 1987.

McManus WF: Infection control and monitoring of the burn wound. Presented at the Annual Meeting of the American Academy of Dermatology, San Antonio, Texas, 5 December 1987.

McManus WF: Treatment of special burns: Chemical, bitumen, and electric. Presented at the Annual Meeting of the American Academy of Dermatology, San Antonio, Texas, 5 December 1987.

Latona PS: Initial management of the burn patient. Presented at the Brooks Aerospace School of Medicine, Brooks Air Force Base, San Antonio, Texas, 9 December 1987.

Latona PS: Initial management of the burn patient. Presented to the Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 10 December 1987.

Keenan JR: Initial management of the burn patient. Presented to the Intensive Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 10 December 1987.

Pruitt BA Jr: Etiology and pathogenesis of inhalation injury. Presented at the Burn Seminar, International Society for Burn Injuries, Denver, Colorado, 10 December 1987.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 17 December 1987.

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Pruitt BA Jr: What's new in surgery: Trauma and burns. **American College of Surgeons Bulletin** 72:56-61, January 1987.

Shirani KZ, Pruitt BA Jr, and Mason AD Jr: The influence of inhalation injury and pneumonia on burn mortality. **Ann Surg** 205(1):82-7, January 1987.

McManus WF: Patterns of infection over the past ten years: Historical patterns. **J Burn Care Rehabil** 8(1):32-4, February 1987.

Burleson DG, Vaughan GK, Mason AD Jr, and Pruitt BA Jr: Flow cytometric measurement of rat lymphocyte subpopulations after burn injury and burn injury with infection. **Arch Surg** 122(2):216-20, February 1987.

Shippee RL and Mason AD Jr: Effect of burn injury on zinc excretion and tissue zinc distribution (abstr 1638). **Fed Proc** 46(3):597, March 1987.

Pruitt BA Jr: Burn treatment for the unburned (editorial). **JAMA** 257(16):2207-8, April 1987.

Vaughan GM and Reiter RJ: The Syrian hamster pineal gland responds to isoproterenol in vivo at night. **Endocrinology** 120(4):1682-4, April 1987.

McManus WF and Jordan BS: Anchoring endotracheal tubes on patients with facial burns. Review from US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas. **J Burn Care Rehabil** 8(3):237, June 1987.

Culbertson GR, McManus AT, Conarro PA, McManus WF, Mason AD Jr, and Pruitt BA Jr: Clinical trial of imipenem/cilastatin in severely burned and infected patients. **Surg Gynecol Obstet** 165(1):25-8, July 1987.

Shimazu T, Yukioka T, Hubbard GB, Langlinais PC, Mason AD Jr, and Pruitt BA Jr: A dose-responsive model of smoke inhalation injury. Severity-related alteration in cardiopulmonary function. **Ann Surg** 206(1):89-98, July 1987.

Pruitt BA Jr: Prospective overview to nutrition/metabolism classic. **Nutrition** 3(6):399-400, December 1987.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Anesthesiology

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 January 1987 - 31 December 1987

INVESTIGATORS

Thomas B. Dougherty, MD, PhD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Anesthesiology

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Jan 87 through 31 Dec 87

INVESTIGATORS: Thomas B. Dougherty, MD, PhD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

During the period of this report, 463 anesthetics were administered to 179 patients, an average of 2.6 anesthetics per patient. The most commonly used anesthetic agent was isoflurane (42.3%) followed by enflurane (27.8%), ketamine (12.3%), narcotics (8.4%), and halothane (6.3%). Due to the nature and combinations of procedures now performed, regional anesthesia is no longer used.

ANESTHESIOLOGY

PREOPERATIVE PROCEDURES

Evaluation. Most burn patients are several days postinjury when first seen by the anesthesiologist. In the immediate postburn period, time is used to gain abundant physiologic data from routine monitoring of various indices, i.e., hematologic (hematocrit, electrolytes, liver and renal function tests), pulmonary (arterial blood gases, respiratory rate, chest roentgenograms), cardiovascular (blood pressure, central venous pressure, cardiac output), and renal (urine output, urine chemistry), in addition to the usual preoperative patient interview and physical examination. All patients, regardless of age, who have electrical injuries are required to have a preoperative electrocardiogram performed to rule out possible myocardial damage.

Preparation. All patients are placed on NPO status after 2400 h the day prior to surgery with the exception of children, who may receive clear liquids up to 5 h prior to surgery. Any patient with an enteral feeding tube, the proximal end of which is shown to be beyond the ligament of Treitz, may have tube feedings continued up to the time of surgery. Due to extraordinary fluid requirements in most burn patients, an intravenous infusion, if not already in place, is begun the evening prior to surgery.

Premedication. Routine medications such as cimetidine and cardiovascular medications are continued up to the time of surgery. Glycopyrrolate (Robinul^{R}), from 0.005 mg/kg to a maximum dose of 0.4 mg/kg, is given intramuscularly 30 min prior to anesthesia or intravenously upon entering the operating room. No other premedications are routinely used with the exception of diazepam preceding ketamine anesthetic.

Fluids. All fluids, except hyperalimentation solutions, are changed to 5% glucose in water or Ringer's lactate upon arrival in the operating room. Hyperalimentation solutions are continued throughout operative procedures.

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of isoflurane, having replaced enflurane which was the primary anesthetic agent for the previous 3 yr. Ketamine, narcotics, and halothane are used, but to a much lesser extent (Table 1).

Isoflurane (Forane^{R}). Isoflurane, which is an isomer of enflurane, is the most recent halogenated ether to be introduced at the Institute. Biotransformation amounts to only

TABLE 1. Pattern of Anesthesia Administration (1984-7)

Agent	1984		1985		1986		1987	
	Number	%	Number	%	Number	%	Number	%
Enflurane	290	62.9	279	71.9	272	66.3	129	27.8
Halothane	0	0.0	2	0.5	35	8.5	29	6.3
Isoflurane	0	0.0	35	9.0	23	5.6	196	42.3
Ketamine	88	19.1	52	13.4	63	15.4	57	12.3
Local	14	3.0	10	2.6	10	2.4	9	1.9
Nitrous oxide	27	5.9	0	0.0	0	0.0	4	0.9
Other*	42	9.1	10	2.6	7	1.7	39	8.4
TOTAL	461	100.0	388	100.0	410	100.0	463	100.0

0.25% of an inhaled dose and no toxic reactions to the metabolic products have been reported to date. Although it has a rather pungent odor that tends to limit its use as a sole mask induction agent, its use in combination with sodium pentobarbital, ketamine, or etomidate provides a smooth anesthetic induction that is significantly more rapid than enflurane plus those three intravenous agents. Isoflurane is presently the most commonly used anesthetic agent at this Institute.

Enflurane (Ethrane[R]). Enflurane is a halogenated ether which provides a relatively smooth anesthetic induction and good muscle relaxation. Biotransformation amounts to 2 to 2.5% of an inhaled dose, which perhaps accounts for the few clinical toxic effects observed. Plasma fluoride levels in hypermetabolic burn patients during and after enflurane administration have been measured and found not to be in the toxic range.

Ketamine. This agent is used both intramuscularly and intravenously to produce its characteristic dissociative state with preservation of basal functions and laryngeal reflexes plus stimulation of the cardiovascular system. Unfortunately, ketamine shares with its parent compound, phenycyclidine, the production of a high incidence of unpleasant hallucinogenic side effects. However, proper patient preparation and premedication with a benzodiazepine appear to have reduced the unpleasant emergence reactions to a level where they are currently of little consideration in the well-selected patient. Laryngospasm, airway obstruction, and regurgitation can occur with ketamine. Pronounced blepharospasm prevents its use in eye cases. All ketamine anesthetics, other than in children, are preceded by intravenous administration of diazepam (0.15-0.2 mg/kg) or midazolam (0.05 mg/kg).

Halothane (Fluothane[R]). Halothane is an halogenated alkane that has met with only limited use over the last 5 yr. Biotransformation can account for as much as 25% of an inhaled dose. Halothane hepatitis, although rare, fortunately has not been reported in burn patients. Since the successful introduction of enflurane and isoflurane, few indications for halothane's use exist in this patient population that may be predisposed to hepatitis from multiple transfusions with blood products. However, its use is indicated primarily in the burned pediatric patient who requires that his airway be secured by an endotracheal tube. Halothane hepatitis has not been reported to be an issue in the pediatric population.

Nitrous Oxide. This agent is used in concentrations of 50-60% with oxygen. It is used mainly in conjunction with other analgesic or anesthetic agents.

Succinylcholine. Succinylcholine has not been used for any purpose at this Institute for more than 11 yr. On the other hand, nondepolarizing muscle relaxants (vecuronium bromide, pancuronium bromide, and atracurium besylate) have been used in 78% of the operative cases over the past year.

Regional Anesthetics. Although regional anesthetics are generally considered one of the safest methods available, its use in the thermally injured patient is limited for several reasons. Sepsis and infection of the skin over or near the site of injection are contraindications for use and multiple-site operations also limit the practicality of this method.

MONITORING TECHNIQUES

Cardiovascular System. Monitoring includes the precordial and/or esophageal stethoscope, peripheral pulse, blood pressure, central venous pressure, Swan-Ganz catheter, electrocardiogram, and urine output.

The Dinamap™ automatic blood pressure cuff is routinely used for intraoperative blood pressure monitoring. Since it can be used over dressings and is noninvasive, it is the most practical method of monitoring blood pressure in our patient population. Usually, blood pressure is monitored at two sites. Direct arterial lines are used when necessary.

Respiratory System. Monitoring includes the rate, auscultation, arterial blood gases, pulmonary functions (pre- and intraoperative), hemoglobin oxygen saturation, and end tidal carbon dioxide. During the past year, the introduction of new noninvasive monitors has made a significant contribution to the management of the thermally injured patient. The measurement of hemoglobin oxygen saturation by pulse oximetry, end-tidal carbon dioxide, and pulmonary function parameters all represent no risk to the patient, are easily obtainable, and are accurate. These monitors have become standard in our anesthetic care of the burn patient.

Body Temperature. Skin, rectal, nasopharyngeal, or esophageal temperatures are continually monitored. Because of the greatly increased evaporative losses in burn patients, hypothermia is a serious problem. Several methods are employed to maintain body temperature during anesthesia. Ambient temperatures were maintained between 85 and 90°F. Maintaining the room temperature above 88°F appears most effective in preventing patient cooling. Anesthetic gases are heated and humidified and radiant heat lamps are used when necessary. Disposable K-thermia™ heating blankets are also helpful and are most effective when used on children. Scrub solutions, intravenous fluids, and blood products are all warmed prior to use.

RESULTS

Complications. There were no intraoperative complications during this reporting period.

Patient Data. Tables 2 and 3 provide overall anesthetic patient data.

Operative Procedures. Table 4 illustrates recent trends in operative procedures.

PRESENTATIONS/PUBLICATIONS

None.

TABLE 2. Use of Selected Intraoperative Monitors (1985-7)

Monitor/Parameter	1985		1986		1987	
	Number	%	Number	%	Number	%
End-tidal carbon dioxide	313	80.7	356	86.8	441	95.2
Inspired oxygen concentration	310	80.0	356	86.8	442	95.4
Temperature	305	78.6	397	96.8	443	95.7
Pulmonary function	277	71.4	332	81.0	391	84.4
Pulse oximeter (hemoglobin saturation)	251	64.7	370	90.2	444	95.9
Arterial line	26	6.7	38	9.3	75	16.2
Central venous pressure	26	6.7	9	2.2	29	6.3
Swan-Ganz catheter	13	3.4	9	2.2	26	5.6

NOTE: Blood pressure and heart rate and rhythm are monitored intraoperatively for every patient. In some patients with toxic epidermal necrolysis, the heart rate and rhythm is ascertained from the blood pressure trace from a sterile aterial line.

TABLE 3. Overall Anesthetic Patient Data (1971-87)

Year	Number of Patients	Number of Patients Anesthetized	% of All Patients	Total Anesthestics Given	Average Anesthetics Per Patients Anesthetized
1987	221	179	81.0	463	2.6
1986	207	143	69.1	410	2.9
1985	197	133	67.5	388	2.9
1984	190	139	73.2	461	3.3
1983	179	98	54.8	291	3.0
1982	231	151	65.4	532	3.5
1981	208	127	61.1	404	3.2
1980	243	148	60.9	531	3.6
1979	267	161	60.3	554	3.4
1978	268	151	56.3	435	2.9
1977	242	129	53.3	344	2.7
1976	277	139	50.2	476	3.4
1975	254	142	55.9	490	3.5
1974	226	123	54.4	380	3.1
1973	273	141	51.7	377	2.7
1972	301	183	60.8	575	3.1
1971	301	179	59.5	475	2.7

TABLE 4. Recent Trends in Operative Procedures (1983-7)

Procedure	1983		1984		1985		1986		1987	
	Number	%	Number	%	Number	%	Number	%	Number	%
Excision	196	42.1	323	41.0	304	43.4	303	38.3	397	44.7
Autograft	203	43.6	371	47.1	304	43.4	372	47.0	389	43.8
Orthopedic	22	4.7	30	3.8	19	2.7	29	3.7	27	3.0
Chrondrectomy	2	0.4	4	0.5	0	0.0	1	0.1	5	0.6
Eye and lid	8	1.7	18	2.3	9	1.3	19	2.4	9	1.0
Intra-abdominal	2	0.4	5	0.6	12	1.7	4	0.5	7	0.8
Plastic	2	0.4	5	0.6	9	1.3	5	0.6	5	0.6
Other	31	6.7	31	3.9	44	6.3	58	7.3	50	5.6
TOTAL	466	100.0	787	100.0	701	100.0	791	100.0	889	100.0

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA315017	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 88 08 16	4. KIND OF SUMMARY D	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	DA	161
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) A Two-Stage Technique Versus a One-Stage Technique for Excision and Grafting: A Prospective Randomized Trial

12. SUBJECT AREAS
06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
88 08	92 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER				
c. TYPE	d. AMOUNT	88	0.1	1
e. KIND OF AWARD	f. CUM/TOTAL	89	0.1	2

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR WAYMACK, J P
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-3411
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) DOUGHERTY, T B
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) SCOTT, C L

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Excision; (U) Skin Grafts; (U) Metabolism; (U) Hemorrhage; (U) Volunteers; (U) Adults; (U) RATI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Early excision followed by immediate skin grafting is widely considered to be a preferred method of burn wound closure. However, the disadvantages of this technique include significant blood loss with need for large volume blood transfusions, lengthy anesthesia time, and excessive metabolic demands. A recently described two-stage technique for excision and grafting of burn wounds allows a period of stabilization, during which blood volume replacement and temperature correction occurs. On the following day, autografting is performed. This study will compare the two-stage excision technique with the conventional one-stage technique in terms of incidence of sepsis, metabolic demands, functional and cosmetic results, duration of hospital stay, and cost in burned soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) One hundred patients with burns requiring excision of > 12% of the total body surface area will be studied. Patients will be randomized into pairs to undergo two-stage or one-stage excision and grafting of their burn wounds. Initial excision of burns will be performed during the first postburn week. A superior result will be considered to be a statistically significant decrease in the number of blood transfusions required, a significant increase in graft "take," a significant increase in the amount of burn excised per procedure, a significant decrease in respiratory complications, a significant decrease in mortality, or a significant decrease in time to wound closure.

25. (U) 8808 - 8809. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board and work will be initiated within the next few weeks.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE — USGPO 1986-491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG6971	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	FB	162
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) 5% Aqueous Sulfamylon Soaks Used in Topical Treatment of Burned Patients

12. SUBJECT AREAS
06 05 Medicine and Medical Research 06 13 Microbiology 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
76 10	99 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
APPROVED BY: Basil A. Pruitt

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER				
c. TYPE	d. AMOUNT	88	1.5	103
e. KIND OF AWARD	f. CUM/TOTAL	89	1.7	130

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR MC MANUS, W F
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-3301
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn Injury; (U) Topical Therapy; (U) Mafenide Acetate; (U) 5% Mafenide Acetate Solution; (U) Volunteers:

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (U) (Continued) (U) Adults; (U) Children; (U) RAII

23. (U) The cause of infection in the wounds of burn patients continues to be a major area of study in order to improve the survival of the severely burned soldier. This study is designed to evaluate the efficacy of 5% aqueous mafenide acetate-soaked dressings, employed either for final debridement of burn wounds or following application of meshed cutaneous autograft to prevent infection and desiccation of the tissue exposed in the interstices of such grafts. A literature search was performed and indicated no duplication of effort.

24. (U) Patients admitted to this Institute for care following thermal, chemical, or electric injury will be treated with 5% aqueous mafenide acetate soaks daily.

25. (U) 8601 - 8612. One hundred and seventy-two patients were treated with 5% aqueous mafenide acetate soaks. Sixteen of these patients exhibited mild cutaneous atopy. This low incidence of mild side effects of 5% aqueous mafenide acetate and its continued clinical effectiveness speak for the continued use of this valuable therapeutic agent.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. © USGPO 1986-491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S62787A874-00, APPLIED RESEARCH

PROJECT TITLE: 5% Aqueous Sulfamylon^{R} Soaks Used in Topical Treatment of Burned Soldiers

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: 5% Aqueous Sulfamylon^{R} Soaks Used in Topical Treatment of Burned Soldiers

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

During this reporting period, 5% aqueous mafenide acetate dressings have continued to be an efficacious treatment modality in the care of the burn wound. Two hundred and seventeen patients were treated with 5% aqueous mafenide acetate dressings, employed either for final debridement of a wound or following application of meshed cutaneous autograft to prevent desiccation of tissue exposed in the interstices of such grafts. An 11.1% incidence of skin rash (atopy) was noted as the only adverse reaction. The clinical results achieved by the use of 5% aqueous mafenide acetate solution strongly support its continued use.

5% AQUEOUS SULFAMYLON[R] SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

During this reporting period, the evaluation of 5% aqueous mafenide acetate solution for topical treatment of the burn wound has continued at this Institute where it was used in 217 of 270 patients (80.4%). The 5% aqueous mafenide acetate-soaked dressings are used as wet-to-dry dressings to debride nonviable tissue elements in preparation for split-thickness autograft procedures or as continuous wet dressings to protect freshly excised wounds that are not autografted. In addition, when meshed cutaneous autografts are applied, dressings are soaked with 5% aqueous mafenide acetate solution to decrease the rate of bacterial growth and to prevent desiccation of tissue exposed in the interstices of such grafts.

Twenty-four patients (11.1%) demonstrated allergic reactions (atopy) with the use of 5% aqueous mafenide acetate solution and these 24 patients demonstrated rapid resolution of the atopic reaction following administration of an antihistamine and/or discontinuation of the 5% aqueous mafenide acetate-soaked dressings. Saline or other aqueous topical antimicrobial agents were substituted when 5% aqueous mafenide acetate-soaked dressings were discontinued and no other adverse reactions were noted in this group of patients.

The use of 5% aqueous mafenide acetate-soaked dressings has continued to be efficacious, both in the preparation of the burn wound for cutaneous autografting and in the prevention of desiccation of ungrafted granulation tissue. In addition, 5% aqueous mafenide acetate solution is most helpful in preventing desiccation or premature bacterial colonization of meshed cutaneous autografts. The dressings over such meshed autografted skin can be left in place for an average of 3 days, allowing development of good adherence of the autografts prior to the first dressing change. The efficacy and the low incidence of adverse side effects speaks for continued use of this solution.

PRESENTATIONS/PUBLICATIONS

McManus WF: Sulfamylon[R]. Presented at the Symposium on Wound Healing, 19th Annual Meeting of the American Burn Association, Washington, DC, 29 April 1987.

Pruitt BA Jr: Use of antimicrobials in burn wound care. Presented at the 81st Annual Scientific Assembly of the Southern Medical Association, San Antonio, Texas, 3 November 1987.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG6970	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	CI	163
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code)
(U) Studies of the Neuroendocrine Abnormalities in Burn Injury

12. SUBJECT AREAS
06 05 Medicine and Medical Research 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
79 10	99 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.7	126
89	1.7	132

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: VAUGHAN, G M
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-5416
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Pineal; (U) Indoles; (U) Catecholamines; (U) Volunteers: (U) Adults; (U) Lab Animals: (U) Hamsters; (U) Rats;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) RAII

23. (U) The objective of this work is to characterize alteration of neuroendocrine function in burned patients in order to improve survival. A literature search was performed and indicated no duplication of effort.

24. (U) To assess photic control of the melatonin rhythm and the daytime loss of sympathetic responsiveness of the pineal will be assessed in murine models for the sympathetic unresponsiveness of critical injury.

25. (U) 8710 - 8809. Actions of the pineal hormone, melatonin, appear to depend partly on the timing of its rhythmic surge. The pineal has been shown to control several hormonal and metabolic functions in nontrauma murine models. Though some of these functions are altered also in severe injury, the interaction of the pineal in injury can only be assessed properly after a better understanding of the control of the pineal itself is obtained. Analysis of pineal N-acetyltransferase and melatonin and serum melatonin in Sprague-Dawley rats adapted to 10 or 14 h of dark per light cycle showed that the nocturnal rise, fall, and acrophase of melatonin synthesis and secretion are controlled by entrainment to lights-on rather than lights-off. The surge depends on beta-adrenergic input to the pineal, with daytime pineal unresponsiveness to catecholamine (particularly in humans and Syrian hamsters) beginning just after lights-on and not due to a daytime fall in beta-receptors. In hamster pineals, activities of two lysosomal enzymes fell abruptly after lights on, indicating a possible role of lysosomes in permitting catecholamine responsiveness. Evaluation of melatonin's role in normal and burned organisms will depend on its accurate measurement. Progress in refining the assay technique (especially difficult in hamster serum) has been made.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: Entrainment of the Rat Serum Melatonin Pattern in Cyclic Lighting

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

George M. Vaughan, MD, Lieutenant Colonel, MC
Russel J. Reiter, PhD*
Basil A. Pruitt, Jr., MD, Colonel, MC

*University of Texas
Health Science Center
7703 Floyd Curl Drive
San Antonio, Texas 78284-7877

ABSTRACT

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Better understanding of the normal pineal system will aid in later assessment of its role in the altered metabolic milieu of burn injury. Though some principles underlying light-dark control of the timing of melatonin production have been elucidated in one strain of rats with use of measurements of activity of an enzyme (N-acetyltransferase (NAT)) as an index of melatonin production, it is not known if these principles apply to other strains or to circulating melatonin itself. In the present studies, Sprague-Dawley and Fischer-344 rats were adapted in either 10 or 14 h of dark per 24-h cycle (same time of middark) and sacrificed at various times. Pineal NAT activity and pineal and serum melatonin all showed a highly significant nocturnal surge. Pinealectomized rats sacrificed at middark exhibited baseline serum melatonin, validating the assay in rat serum. In intact rats of either strain, the time of the fall in NAT and pineal and serum melatonin was delayed about 2 h in the longer dark condition with a 2-h delay of the time of lights-on, despite the 2-h advance in lights-off. In Fischer rats, the time of rise of these variables was the same in both photic conditions. In Sprague-Dawley rats, the rise in these variables (significantly for serum melatonin) appeared to be delayed in the longer dark condition, again despite the earlier lights-off. The results conform to the two-oscillator model of photic control of melatonin production described in Wistar rats in which evening and morning components of the endogenous pacemaker (controlling the rise and fall of melatonin synthesis, respectively) are entrained by the timing of lights-on. A contribution of the timing of lights-off (in these photic conditions) upon the evening component (rise of melatonin) was more evident in the Fischer rats, with the earlier lights-off apparently neutralizing the effect of later lights-on. The results do not support the alternative clock-gate model. They do suggest that predictions from the two-oscillator model can be extended to other rat strains. That the patterns observed also include circulating melatonin

indicate that the entraining effects of light can control the pattern of exposure of tissues to melatonin. This suggests the possibility of melatonin's interaction at endocrine target sites should be investigated in burn injury.

ENTRAINMENT OF THE RAT SERUM MELATONIN PATTERN IN CYCLIC LIGHTING

Melatonin (MEL), the best hormonal indicator of the function of the biological clock in humans (1), is also a potent regulator of the reproductive and thyroidal axes in other mammals (2-10), and its production in the pineal gland is regulated by the sympathetic nervous system (1,11). Although major alterations in sympathetic, gonadal, and thyroidal function are characteristic of burn injury, observations of postburn patterns of endogenous MEL (12) and of other hormonal variables after pinealectomy in burns (13) have not fit a model based on simple relationships predicted by the conventional assumption of elevated sympathetic activity stimulating MEL secretion which might depress gonadal and thyroidal function. This likely results partly from incomplete understanding of the factors that enter into what is increasingly recognized as a complex set of interrelationships in the pineal system (14). The nocturnal MEL surge is depressed in burned humans and not greatly altered in burned rodents (12). It is becoming clear that light-dark responsiveness of the MEL pacemaker, pineal responsiveness to catecholamines, and hypothalamic responsiveness to MEL are all complex rhythms, with entrainment of one component apparently having effects on others seen over intervals of time.

In this study, we have focused on what might be considered the initial events in the function of the pineal axis, involving the entraining influence of cyclic lighting on MEL synthesis. So far, most work with rats in this area has been done in the Wistar strain (15-20), with use of measurements of pineal activity of N-acetyltransferase (NAT), the enzyme (in the biosynthetic pathway for MEL) whose changes are usually reflected by changes in pineal MEL content, as an index of the pineal MEL rhythm. Those studies have identified evening and morning responses to light striking the eyes, indicating the presence of what might be conceptualized as evening and morning oscillators (symbolized by E and M) being light-sensitive but endogenously functional components of the circadian pacemaker presumably in the hypothalamus controlling the onset and fall, respectively, of pineal MEL synthesis. The timing of E and M activation define the duration of the nocturnal surge of NAT activity in Wistar rats. E and M as used herein refer either to the two oscillators which cannot be observed directly, or to the rise and fall of NAT or MEL, which can be observed. The term "oscillators" is used to signify that functionally they appear to activate cyclically even without light in constant darkness, with a periodicity near 24 h. They can be entrained by light to activate at specific times in the 24-h cycle somewhat independently of each other.

A productive approach in the above mentioned studies was to adapt Wistar rats chronically to a certain length of the dark phase (scotoperiod) in the 24-h light-dark (LD) cycle, then expose them to a novel 1-min light pulse during one night, applied once at different times in separate studies during a regular dark phase, and then to leave the rats in constant darkness (without turning the lights on at the usual time) for one or more days. Then the animals were sacrificed and pineal NAT activity was measured to define the change in clock time for the rise and fall of NAT, indicating the phase-resetting of E and M brought about by the light pulse (16,17,19). In an LD cycle with a long dark phase of, e.g., 18 h, a light pulse near the beginning of the scotoperiod delayed E and M; one near the end of darkness advanced E and M, though the effect on E was small.

The phase response curves (developed separately for E and M and separately for varying lengths of the scotoperiod), relating the timing of the novel light pulse (within the scotoperiod) to the magnitude and direction of the subsequent E or M phase shift, and the areas circumscribed by the curves revealed that: (1) a pulse early in dark slightly more effectively delayed E than M; a pulse late in the usual dark phase more effectively advanced M than E; and a pulse at times in between would shift one but not the other, or shift them oppositely, depending on the timing of the pulse, the length of the scotoperiod, and the number of day/night cycle equivalents elapsing (in darkness) between the night of the light pulse and the NAT measurements; (2) in the shorter scotoperiods (12 or 6 h), a light pulse would not advance E even when applied late in the scotoperiod; (3) with chronic adaptation to 24-h cycles having progressively shorter (< 18 h) dark phase lengths, the sensitivity of E and M to resetting by a light pulse was reduced (less phase difference from unpulsed animals); (4) with shorter entraining dark phases, the timing of the periods of sensitivity to a light pulse also changed; the late dark times of light-induced phase advancement (of M) moved closer to early dark times of light-induced phase-delay (seen best in E) with resulting compression of the E-M interval in terms of the timing of when they are sensitive to further shifting (resetting) of oscillator phasing by a single light pulse in the dark phase (it is noted that their times of activity - reflected in the times of NAT rise and fall before a pulse is applied - are already closer together in a shorter dark phase just from chronic adaptation to the times of light presence before lights-off and after lights on in the particular LD condition); (5) the compression of E and M sensitive times in chronically shorter dark phases resulted from the late dark time of light-pulse-induced advancement moving in the direction toward middark, with little or no change in the early dark time of pulse-induced phase delay in which the peak magnitude of phase delay response occurred with light pulses relatively late (near middark) in long dark lengths; (6) longer scotoperiod, though, allowed greater magnitudes, particularly of phase

delays, to be induced by a light pulse, suggesting that on a chronically short scotoperiod the phase delay already produced by light at the end of the light phase leaves less possibility for more phase delay from an early dark phase light pulse (and vice versa for the previously recurring light at the beginning of the light phase, such that a late dark phase light pulse produces less additional phase advance). This diminution of light pulse effectiveness in short scotoperiods may be due to either an interaction between E and M as their functions come closer together, providing resistance to stimuli inducing further closure of the E-M interval; or to an effect of light at the end of the light phase, tending to delay the setting of E and M and partially neutralizing an advancement after a late dark phase light pulse, and a similar but opposite effect of light at the beginning of the light phase tending to advance E and M and offsetting some of the phase delay from an early dark light pulse; this mutual opposition of phase-modifying effects of light at the ends of the dark phase being more prominent with shorter scotoperiod; (7) despite the possibility of an interaction between E and M which may tend to oppose further movement toward one another in short scotoperiods, E and M respond to light pulses as if there were little or no interaction between them to explain a shift in the time setting of one in the same direction of a shift in the setting of the other, i.e., the phase-shifting response of one does not necessarily depend on "drag" from the response of the other - thus E and M can function to a certain extent independently; (8) the time course for expression of a light pulse phase shifting effect on E and M differ--in LD 12:12, an early dark phase light pulse (after immediately suppressing NAT) then delayed E but not M on the same night; whereas, both were delayed on subsequent nights; a late dark phase pulse advanced M (but not E) on the next cycle, though both were advanced by four cycles later (19) - this further illustrates the relative independence of E and M.

The above studies show that the time position and length of the nocturnal NAT surge depend on the photically determined setting of two functional components, E and M, of the pacemaker, to begin and terminate, respectively, the NAT surge. Both components oscillate (trigger their NAT response once per LD cycle) endogenously but are entrained to their relative position in the dark phase by preceding adaptation to the ambient LD cycle and can be reset by novel exposure to one pulse of light delivered toward the beginning or end of the dark phase. Thus, without the novel light pulse, exposure to a given LD cycle entrains E to activate after lights-off and M to activate before lights-on, confining the duration of the NAT surge entirely to the given dark phase as experienced in the preceding cycles. Further, from what was reviewed above, on cycles with shorter dark phases (6-12 h), light both at the end and the beginning of the light phase (near the times of lights-off and lights-on) would be expected to contribute to the setting of E and M, and in longer dark phases light at the

beginning of the light phase (i.e., near the time of lights-on) should be the predominant influence on the setting of E and M.

In fact, exposing Wistar rats to a chronic entraining scotoperiod length that varied between studies, such that in effect the scotoperiod was lengthened symmetrically about the middark point, showed that as the dark phase was lengthened in increments above 6 h duration, the onset of NAT activity (activation of E) was advanced. However, with darkness > 12 h, there was no further advancement of E. Instead, in some studies, rats exposed to 18 h of darkness had even a delay in E back beyond the time of E in 12 h darkness toward the time of E in 8 h darkness (19). The timing of M was consistently delayed with greater increments in dark phase length up to 18 h of darkness (19). The magnitudes of the E and M shifts were such that the duration of the NAT surge increased steadily from 4 to 10 h in length as the duration of darkness increased from 6 to 18 h in length. The animals had entrained to each length of darkness. The interval between lights-off and the NAT rise was 2-3 h and increased only with dark phase lengths > 12 h (to 7 h in 18 h of darkness), and the interval between the NAT decline and lights-on became perceptible in 8 h of darkness and remained 1-2 h in longer darkness. Hence, as dark phase length increased, with adaptation in each length, the NAT surge expanded, occupying principally the latter part of long dark phases. These observed relationships with use of differing LD cycles after entrainment together with those from the light pulse studies indicated that both the rise (E) and the decline (M) are entrained by light at the borders of the light phase (but mainly by light at the beginning of the light phase in cycles with long scotoperiods).

Since light exposure to the eyes immediately and persistently suppresses pineal NAT activity as long as the light is on, from one point of view daytime light may be taken as a gating influence, restricting the NAT rise to the dark phase when the gate is open and when, also, after entrainment a single internal clock is set to raise pineal NAT (clock on) during darkness. According to this "clock-gate" model (21,22), in shorter scotoperiods there may be narrower gating, and in longer scotoperiods less or no gating. This model has been suggested as an alternative to the two-oscillator model of Illnerova discussed above. The clock-gate model postulates that a single clock tends to be on or off (perhaps 12 h each) for the same duration regardless of the length of the light or dark phase in 24-h cycles. Shorter scotoperiods would shorten the duration of high nocturnal NAT activity only by the longer light phase with its action of gating down the NAT duration, eliminating the front part of the surge, i.e., the suppressive action of light at the end of the light phase would prevent NAT from rising until after a constant lag time (even though the clock had already come on during light). The clock then goes off (and NAT falls) always before the end of the dark phase. This model says that when the length of the light phase is

having an effect to delay the NAT rise past where it would otherwise be, this effect is due only to suppression of NAT, but is not due to keeping a functional component (oscillator E) entrained to be set to raise NAT at the observed time. The NAT clock has simply come on and will not be expressed until after the light goes off.

The studies from Illnerova's laboratory (15-20) have shown that this is apparently not the case. Those with novel light pulses were designed to include acute extension of a dark phase through subsequent cycles, such that at the time of NAT measurement, any suppressive effect of daytime light was not present. No evidence was found for an NAT clock being in the "on" position with its expression suppressed late in the light phase. Even with an entraining dark phase as short as 6 h and then novel failure to turn the lights on (and in this case with no novel light pulse), after one cycle the circadian time of NAT rise was not affected, though the time of the fall was delayed. The other studies showed that not only had the time of the rise and fall (E and M) been entrained by the light of the photoperiod, but that E and M times could be manipulated somewhat independently (15-20).

The novel presence of light during a time of sensitivity tends to reset the oscillators relatively quickly (20), and novel absence of light during such a time allows the oscillators to reset more gradually over time. Thus, sudden extension of the dark phase only into the evening light time allowed the NAT rise to advance by increments over the next 6 cycles in Wistar rats and the NAT fall to advance by much smaller increments over this time and in humans allowed the plasma melatonin rise to advance more by the seventh than by the first subsequent cycle (22). In Wistar rats, the pattern is markedly altered (diminished daily E advancement and now daily M delay) by simultaneous sudden expansion of the dark phase also into the morning. These results and the patterns seen after sudden expansion of dark only into the morning (greater daily delay of M than of E) (18), together with the observations after light pulses and separately after entrainment to varying lengths of darkness (15-17,19,20) clearly show that both the rise (E) and the fall (M) of NAT are entrained to occur at the time at which they are observed to occur in Wistar rats in entraining LD cycles almost regardless of the brevity of the dark phase. An exception may be cycles with a dark phase < 6 h; wherein, some data suggested the possibility that such extreme compression of the E-M interval may not be attainable and the suppressive effect of light (rather than an entrained M) might dictate the NAT fall in such an extreme condition (19). Otherwise, both E and M are entrained, and at least in Wistar rats, there is no NAT clock in the "on" mode prior to the time of the NAT rise (E activation), despite many adequate attempts that would have allowed such to be manifested.

Though the clock-gate model does not explain the observations in Wistar rats, "suppression" of the early part of the NAT surge by suddenly longer (evening extension of) light may be viewed possibly as a kind of gating. However, this "suppression" of NAT is brought about by resetting (entraining) the E oscillator activation to a later time for the NAT rise; or, if the light extension is long enough (11-h extension in LD 12:12) E activation (NAT rise) is prevented (20).

The overall results to date suggest the two-oscillator model as an appropriate frame of reference in Wistar rats for photic control of the nocturnal surge of melatonin synthesis, based on pineal NAT measurements. However, it is necessary to determine if observations predicted by this model can be obtained in other strains and if such observations apply to actual circulating melatonin, previously only assumed.

MATERIALS AND METHODS

Male rats of two albino strains, Sprague-Dawley (SD) and Fischer-344 (F), were obtained after weaning and placed two per clear plastic cage with fragmented corncob bedding in an ambient temperature of 22°C with food and water always available. Fluorescent ("cool white") lighting (averaging 60 foot candles intensity at cage fronts) was present for either 14 or 10 h each day in two respective separate rooms. This represented dark phase lengths of either 10 or 14 h, and LD 14:10 (lights off 2000 h, on 0600 h) or 10:14 (lights off 1800 h, on 0800 h), respectively. In either case, middark was at 0100 h. After 8 wk in either lighting condition, blood and pineals were taken after guillotine decapitation. Sacrifice times were at 1300, 2000, 2200, 0100, 0400, 0600, and 0800 h in both lighting regimens, and at 1800 h in LD 10:14. Sacrifices nominally at the light-dark or dark-light transition were performed a few minutes before the transition. Additional groups, pinealectomized (Px) 8 wk earlier, were sacrificed at 0100 and 1300 h in the long dark (LD 10:14) regimen. For a given strain, light regimen, and time of sacrifice, group n was 7-9 rats (Px groups 5 each). Sacrifice in darkness was under dim red lights (15-W incandescent bulbs, Kodak 1A filters).

Serum was saved at -60°C for melatonin assay (5) with the Rollag antibody after chloroform extraction, and washes with HCl, NaOH, and H_2O, and two petroleum ether washes of the aqueous eluate (least detectable 5 pg/ml). Heads were brought to a lighted necropsy room where the pineal was immediately removed and frozen individually in tubes on solid CO_2 and transferred to -60°C until homogenization in buffer for determination of N-acetyltransferase (NAT) activity (23) and melatonin content (24).

Between-group statistical comparisons were made by Bonferroni-corrected t tests. Overall comparisons between lighting regimens were made by two-way analysis of covariance,

partitioning variance between light regimen type and time of sacrifice for time points common to both regimens. Variables were analyzed within a lighting regimen by cosinor periodic function (sine wave) regression for significance of a rhythm (amplitude > 0) and description of its timing (acrophase, time of best-fit curve peak) with use of time data as two independent variables after transformation into both the sine and cosine of the time-angle on the basis of 24-h periodicity (24 h = 360°). The resultant sine and cosine coefficients (as rectangular coordinates) were converted to polar coordinates to give the acrophase and amplitude. Comparisons of timing (acrophase) of fluctuation were made for a given variable between lighting regimens (if the rhythm was significant in both) without interference by parameters of amplitude and mesor (overall average for a light regimen), utilizing data from time points common to both regimens. Cosine and sine coefficients (as rectangular coordinates for an acrophase point) and their standard errors (SE) determined from a regression separately in each of the two photic regimens were used to compute a SE ellipse for 36 points about the acrophase point in the respective condition. With expression of these points as polar coordinates and use only of the angle coordinates, the central acrophase angle and its one-SE angular variation on either side were described for a particular variable separately in both photic regimens. Both acrophases and their SE (each SE taken in the direction of the other acrophase) were used in t tests (with the residual degrees of freedom from the regressions that computed the cosine and sine coefficients and their SE) to compare the nocturnal acrophases between the two photic conditions. The statistical analyses were made using the P1D, P7D, and P1R programs of the BMDP package on a VAX 11/780 computer (25).

RESULTS

Table 1 shows that the 24-h rhythms of pineal NAT and melatonin and serum melatonin were highly significant in nonpinealectomized rats of both strains in both photic regimens. The delay in acrophase in long (vs. short) dark was significant for pineal and serum melatonin in SD rats and for serum melatonin in F rats. In Figure 1, the generation of what appears graphically or statistically as phase shifts can be seen as group differences at various time points between photic conditions. Of note, for SD rats, the delayed rise of serum MEL and delayed fall of pineal NAT and melatonin and serum melatonin in long (vs. short) dark were significant. For F rats, the timing of the rises appeared unchanged between photic conditions; whereas, the fall of all three variables was delayed in long (vs. short) dark. Prior pinealectomy prevented the nocturnal rise in serum melatonin in both strains, as observed in measurements at about middark in LD 10:14.

Figure 2 shows for intact rats the relative positions of the acrophases between photic conditions, together with their

TABLE 1. Cyclic Parameters from 24-h Periodic Regression of Melatonin Variables

Strain	Variable	L:D	24-h Mean	Amplitude	Acrophase	Acrophase Difference
Sprague-Dawley	Pineal NAT	14:10	1.94	2.89*	02.45	
		10:14	2.17	3.04*	04.04	01.59
	Pineal Melatonin	14:10	1.03	1.25*	01.70	
		10:14	1.24	1.40*	03.34	01.64**
	Serum Melatonin	14:10	29.2	29.8*	01.68	
		10:14	34.5	37.4*	03.65	01.98***
Fischer-344	Pineal NAT	14:10	0.94	1.28*	02.06	
		10:14	1.19**	1.55*	03.35	01.29
	Pineal Melatonin	14:10	1.07	1.24*	01.28	
		10:14	1.32	1.47*	02.55	01.27**
	Serum Melatonin	14:10	36.1	35.4*	01.97	
		10:14	42.1	40.9*	03.33	01.37**

NAT, N-acetyltransferase; *P < 0.001, significance of the rhythm (vs. zero amplitude); **P < 0.05; ***P < 0.01, LD 10:14 vs. 14:10.

The 24-h mean and amplitude are in units for the respective variable (see Fig 1), and the acrophase (time of cosinor curve peak) and its difference between photoperiodic conditions (last column) are in decimal hours.

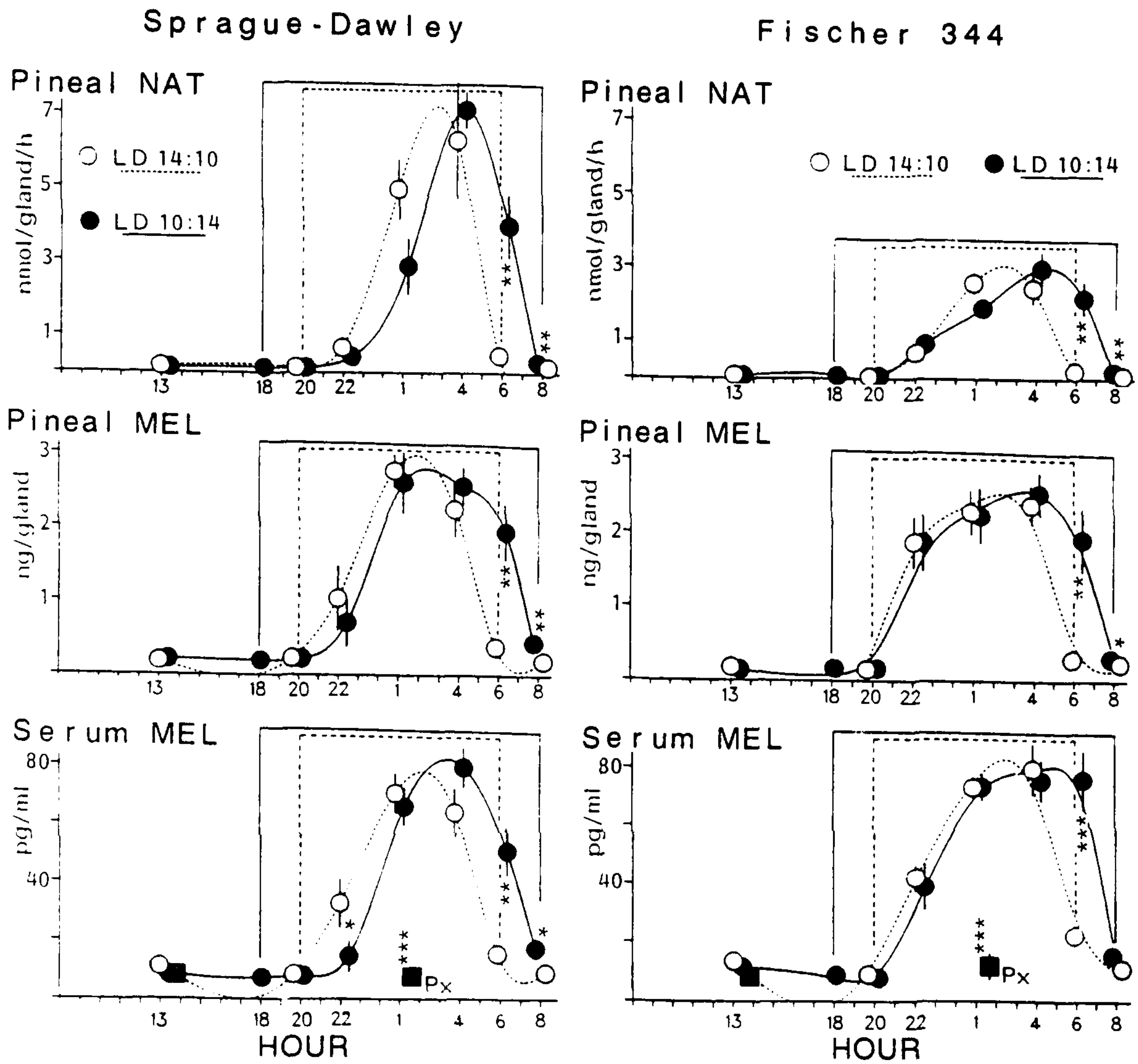

FIGURE 1. Melatonin variables (mean ± SE) in each time group. The vertical lines connected at their tops define the lengths of the dark phase, different between LD 14:10 (dashed) and 10:14 (continuous). The closed squares (serum melatonin) represent pinealectomized (Px) rats in LD 10:14. The curved lines represent spline fits to the means. NAT, N-acetyltransferase activity, a measure of pineal melatonin synthesis; MEL, melatonin as content in the pineal or concentration in serum. *P < 0.05, **P < 0.01, ***P < 0.001, LD 10:14 vs. 14:10, or Px vs. the other groups, at the particular time point.

95% confidence ellipses. The distance from the origin represents the amplitude of the rhythm as maximum excursion of the best fit cosinor curve above the curve average. Failure of the ellipses to include the origin indicates significance of

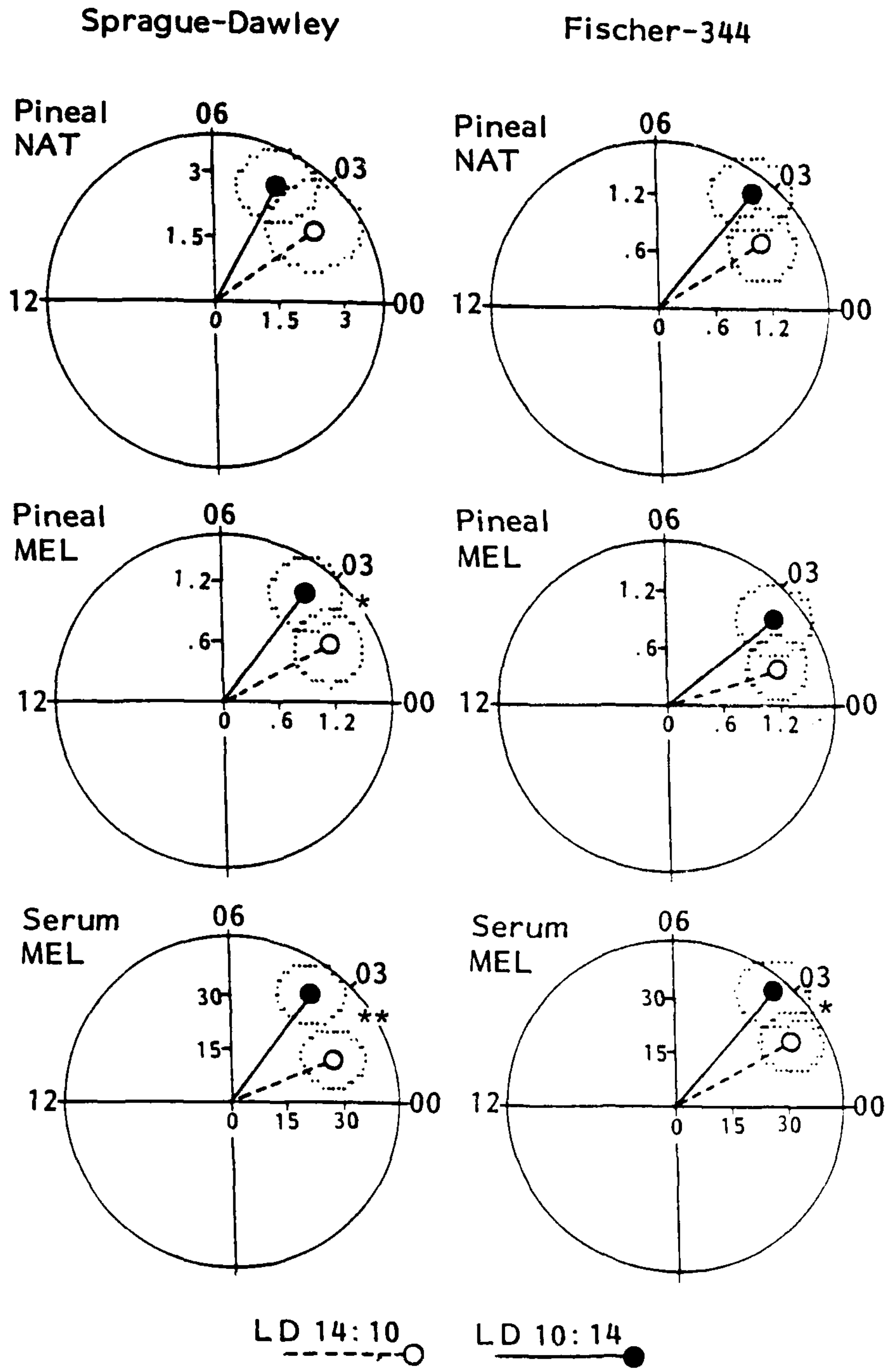

FIGURE 2. Polar depiction of amplitude (distance from origin) and acrophase (angle from midnight 00 in h marked on the circle) shown as a single point, for melatonin variables. The 95% confidence ellipse of the central point is shown by small dots. $*P < 0.05$, $**P < 0.01$, LD 14:10 vs. 10:14 (with respect only to acrophase).

the rhythm. The overall apparent serum melatonin acrophase delay in long (vs. short) dark was due to a delayed decline of the nocturnal surge in F rats and to a delay of both rise and decline in SD rats (as seen in Fig 1). In the latter case, the delay was close to 2 h (as noted in Table 1), the magnitude of the delay in the time of lights-on.

DISCUSSION

Work in Wistar rats (15-20) has shown that the morning fall of pineal melatonin synthesis before the onset of light (M oscillator activation), indexed as the decline in NAT activity, is entrained mainly by lights-on. Some additional influence on M timing may be exerted by the timing of lights-off, particularly when the entraining dark period is short (i.e., when the entraining LD cycle has a relatively short dark period). The evening rise in NAT after the onset of darkness (E oscillator activation) may also be entrained mainly by lights-on if the dark phase is long (> 12 h), in which case the NAT rise (E) seems to occur at a time also determined in part by a resistance to compression or narrowing between the E and M times of activation. This results in a tendency for expansion of the NAT surge duration (E-M interval). As the entraining dark phase shortens (particularly at <12 h), bringing lights-off to later times, lights-off begins to have an influence in overcoming the tendency for E activation to be set as early as it would be by lights-on and the tendency for expansion between E and M activation times. A smaller such effect of lights-off on M can also be seen.

The overall result of the above is that in comparing between two entraining dark phase lengths (one symmetrically expanded by a few hours), the direction of change in NAT rise time (E) depends on the general magnitude of the dark phase lengths: E advances (is earlier) in the longer dark when both dark phases are relatively short, but E delays (is later) in the longer dark when both are relatively long. In contrast, the direction of change in M with longer dark phases does not depend much, if any, on the general magnitude of compared dark phase lengths, being delayed in dark phase lengths with further symmetrical increases above 8 h duration.

The present finding of relatively delayed fall of melatonin variables in longer dark in both SD and F rats conforms to the pattern of control of the M oscillator through entrainment by lights-on previously described in Wistar rats. Lack of advancement of the rise in melatonin variables in longer dark for both SD and F rats also conform to the results in Wistar rats. For purposes of comparing among various symmetrically increasing lengths of an entraining dark phase, Wistar rats were studied in 6, 8, 12, 16, or 18 h of darkness duration (19). The earliest NAT rise was in 12 h dark, and interpolation between curves indicates that the rise of NAT in 14 h dark would be simultaneous with or only a little in

advance of that expected for 10 h dark. Thus, our similar time of melatonin rise for 10 and 14 h of dark in F rats suggests a zero net effect of the influence of the alteration in lights-off opposing that of the alteration of lights-on for E activation, like that gleaned from similar curves in Wistar rats. For our SD rats, it appears that the influence of lights-off on E, at these dark phase lengths, is not manifested, suggesting that the tendency for expansion of the E-M interval is not as great in the SD rats (and thus not being compressed in 10 h of darkness), which allows the onset of the serum melatonin rise (reflecting the E oscillator) to delay in response to a delayed lights-on, despite an earlier lights-off.

As part of a study of the effects of bright light 2 h before and after sleep on the evening rise of human plasma melatonin in psychiatric depression (26), the results in the normal control subjects receiving bright light both before and after sleep are of interest. Sleep was 2200-0600 h. Without the bright light at 2000-2200 h and at 0600-0800 h, there was only dim light, which was analogized to dark because human melatonin can rise in dim light, and bright light is required to suppress human plasma melatonin at night. Thus, it might be possible to consider the double light treatment as defining a scotoperiod of 8 h for the week of the test, with the prior baseline condition of effective "darkness" perhaps symmetrically enlarged, presumably by 4 h, though that was indeterminate because of absence of defined bright lighting in the baseline condition. Melatonin onset was delayed after a week of bright lighting, tantamount to an advancement of melatonin onset in expanded darkness, opposite to our finding of delayed E in SD rats. This suggests either of two explanations: (1) in the baseline condition, morning light was less dim than evening, such that with light treatment the main difference was extra light in the evening, maximizing the effect of lights-off in entraining M in a relatively short scotoperiod, as seen in Wistar rats with advancement of the NAT rise when only lights-off was advanced, after prior entrainment to 8 h of darkness (18); or (2) light treatment provided an effective photic alteration from baseline at both morning and evening, and the 8-h experimental dark phase length (scotoperiod) was short enough to provide pressure to expand the E-M interval and advance E in the baseline (longer effective dark) condition. This would give the pattern of entrainment of E in part by lights-off, similar to the NAT pattern in Wistar rats upon symmetrical expansion of darkness above 8 h (to 12 H duration) (19).

The present studies indicate that photic control of melatonin production in rats, conforming to the two-oscillator model of Illnerova (15-20), is expressed in circulating melatonin levels. This suggests the possibility that the intricate pattern of photic control over the circadian architecture of pineal function can be transmitted to melatonin target tissues. Further, the pattern of photic response among

rat strains is quite similar, though some difference in control of the evening oscillator (relatively more control by lights-on) may be present in SD rats.

An additional gain from these studies is that the observed patterns even in circulating melatonin do not support the clock-gate model for control of pineal melatonin production. In this model, end-light-phase light would suppress melatonin production in LD 14:10 even though the melatonin "clock" was "on", and account for the timing of the melatonin rise in LD 14:10. The earlier lights-off in LD 10:14 thus should have allowed melatonin production to rise earlier; however, it did not. In SD rats, the rise was actually later in LD 10:14. Thus, with use of these photoperiods (LD 14:10 and 10:14) in two strains of rats, any suppressant (nonentraining) action of end-light-phase light (as part of the clock-gate model) apparently did not account for the observations. This interpretation is consistent with earlier observations of NAT in Wistar rats (15-20) demonstrating entrainment of both the rise (E) and fall (M) of melatonin production by light, without a single melatonin "clock" being in the "on" position prior to the time of activation of E, in dark phase lengths as short as 6-8 h.

PRESENTATIONS

Vaughan GM: Entrainment of both rise and fall of rat serum melatonin in cycling lighting. Presented at the 70th Annual Meeting of the Endocrine Society, New Orleans, Louisiana, 9 June 1988.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA314707	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
88 06 06	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	DA	165
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) The Effect of Recombinant Human Growth Hormone Treatment on the Rate of Healing on Burn Patients Who Require Skin Grafting

12. SUBJECT AREAS

06 05 Medicine and Medical Research 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
88 05	92 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

a. DATE EFFECTIVE APPROVED BY

b. CONTRACT/GRANT NUMBER

c. TYPE

d. AMOUNT

e. KIND OF AWARD

f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	c. FUNDS (In thousands)
88	0.3	5
89	0.4	7

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): MC MANUS, W F
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available): VAUGHAN, G M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns; (U) Peptides; (U) Growth Hormones; (U) Wound Healing; (U) Pharmacology; (U) Volunteers; (U) Adults; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) This double-blind, randomized, placebo-controlled study is designed to evaluate the efficacy and safety of recombinant human growth hormone on the rate of wound healing in burned patients. A literature search was performed and indicated no duplication of effort.

24. (U) Up to 100 patients will be entered into this study. Each morning (beginning on the day of the first graft operation), the patients will receive 10 mg recombinant human growth hormone, 5 mg recombinant human growth hormone, or reconstituted placebo excipient by subcutaneous injection. Treatment will continue for the duration of hospitalization. The rate of healing of donor sites will be measured by daily inspections and computerized planimetry based upon photographs taken every other day. The three groups will then be compared.

25. (U) 8805 - 8809. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board. Two patients have been enrolled in the study during this reporting period. Upon enrollment of 25 patients, the data will be analyzed for the effect of recombinant human growth hormone on wound healing in thermally injured patients.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE * USGPO 1986 -491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: The Effect of Recombinant Human Growth Hormone Treatment on the Rate of Healing in Burn Patients Who Require Skin Grafting

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Barry M. Sherman, MD*
Douglas W. Wilmore, MD**

*Genetech, Inc.
South San Francisco, California 94080

**Harvard Medical School and
Brigham and Women's Hospital
Boston, Massachusetts 02115

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: The Effect of Recombinant Human Growth Hormone Treatment on the Rate of Healing in Burn Patients Who Require Skin Grafting

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Barry M. Sherman, MD
Douglas W. Wilmore, MD

Investigators have attempted to modify the metabolic response to stress with hormonal interventions. Administration of insulin and anabolic steroids have met with little success. Growth hormone administration has been shown to have a positive effect on nitrogen balance in healthy adults. The current availability of biosynthetically produced human growth hormone has made it possible to explore the efficacy of this hormone in promoting wound healing in thermally injured patients. This study was designed as a double-blinded, randomized, placebo-controlled study to determine whether administration of recombinant human growth hormone accelerates wound healing in thermally injured patients. At the end of this reporting period, 3 patients from this Institute had been enrolled in the study. Upon enrollment of 100 patients, data will be analyzed for the effect of recombinant human growth hormone on wound healing.

THE EFFECT OF RECOMBINANT HUMAN GROWTH HORMONE TREATMENT ON THE RATE OF HEALING ON BURN PATIENTS WHO REQUIRE SKIN GRAFTING

The development of genetic enegineering techniques has made available large amounts of naturally occuring peptides. It is now possible to test the potential of such peptides, some of which may be clinically useful in situations which require tissue regeneration. This study is designed to evaluate the efficacy and safety of recombinant human growth hormone on the rate of healing of burn patients.

The overall effect of growth hormone on protein metabolism is illustrated by the well documented increase in linear growth that results from the administration of growth hormone to growth hormone-deficient children. Growth hormone administration improves nitrogen balance, increases somatomedin-C levels, and increases body cell mass in growth hormone-deficient children (1). Growth hormone also exerts a positive effect on nitrogen balance and somatomedin-C in healthy adults (2-4).

Recent studies using methionyl human growth hormone (Protropin®) in calorie-deprived human volunteers illustrated its anabolic effect. Hypocaloric parenteral nutrition resulted in negative balance of nitrogen and potassium. These trends were markedly reversed when growth hormone was given. Changes in body weight followed a similar pattern (5).

Stressed patients, whether septic, postoperative, or victims of burns or trauma, undergo well described metabolic changes that result in negative nitrogen balance and loss of body protein. There is evidence that as the catabolic state continues, it results in decreased resistance to infection, poor wound healing, and in general, prolonged recovery. When patients cannot eat, nutrients may be provided by intravenous feedings, but such an approach is not always successful and parenteral nutrition is associated with significant side effects.

Investigators have attempted to modify the metabolic response to stress with hormonal interventions, notably insulin (6), anabolic steroids (7-8), and growth hormone. Administration of insulin and anabolic steroids has met with little success and has not been generally used in clinical practice. Until recently, growth hormone was not available in sufficient quantity for use as an anabolic agent. The current availability of biosynthetically produced human growth hormone has made it possible to explore the efficacy of this hormone on clinical indications.

A limited number of studies were carried out with pituitary growth hormone, primarily in burned patients and experimental animals. Gump et al (9) demonstrated that burned rats receiving adequate nutrition and growth hormone did not suffer a catabolic response, but when the burned rats were starved, they lost weight at a greater rate than control animals. In 1960, Soroff et al (10) demonstrated similar positive effects of human growth hormone administration in patients during the anabolic phase of burn recovery.

Liljedahl et al (11) and Wilmore et al (12) both showed that growth hormone caused a significant improvement in nitrogen and potassium balance in the postburn period, the latter specifically with high calorie and protein intake.

The role of growth hormone in postoperative nutrition and nitrogen loss has been examined in a few studies. Rowe and Kinney (13) demonstrated an alteration in substrate utilization in postoperative orthopedic patients given growth hormone, with a fall in respiratory quotient and a shift to lipid substrate. Johnston and Hadden (14) showed no improvement in nitrogen balance after herniorrhaphy in patients treated with growth hormone compared to matched controls. However, nitrogen intakes were low, caloric provision was not measured, and only the immediate postoperative period was studied.

In recent work, Wilmore et al used methionyl human growth hormone (Protropin®) to evaluate whether growth hormone can promote anabolism in surgical patients. Patients (n=9) received a constant parenteral infusion of a hypocaloric diet which provided 1100 kcal/24 h and 1.3 g/kg/24 h protein for at least 2 wk. During one week, 10 mg Protropin® was given subcutaneously daily and the other week served as the control. Daily balance studies demonstrated that growth hormone resulted in significant retention of nitrogen (+3.4 g/24 h) and phosphorus (+218 mg/24 h) despite provision of only 60% of caloric requirements. Six patients received Protropin® daily (10 mg SC) for up to 25 consecutive days. Significant nitrogen and phosphorus retention occurred over the entire period of growth hormone administration and no significant side effects were observed.

It was the clinical impression of these investigators that the nitrogen retention associated with growth hormone administration was accompanied by acclerated wound healing and an apparent decrease in morbidity and hospitalization time.

It was the purpose of this double-blind, randomized, placebo-controlled study to determine whether administration of recombinant human growth hormone accelerates wound healing in burned patients. Because the heterogeneity of burns makes it difficult to evaluate healing among patients, this controlled study focuses on the rate of healing of the patients' donor

graft sites. However, healing of the primary burn, duration of hospitalization, and mortality will also be evaluated.

MATERIALS AND METHODS

Study Design. This is a multicenter, randomized, double-blind study. Patients are randomized to receive daily subcutaneous injections of either 5 or 10 mg recombinant human growth hormone or placebo until the end of hospitalization. Patients are randomized to treatment or control groups by the Biostatistics and Data Management Division of Genentech, Inc. (South San Francisco CA). Groups are balanced for age, cause, and extent of burn.

The "study wound" is a donor site where skin is taken for grafting. The donor site is part of a planned and necessary surgical procedure and the care of the donor skin is not greatly altered from standard techniques. The methods for taking the skin and caring for the donor site wound are standardized for all patients. All wounds are inspected and evaluated by one observer.

Number of Patients. Up to 100 patients will be entered into this study based on eligibility criteria and informed consent. For purposes of computing statistical power, an average healing time of 12 days for "young" patients and 16 days for "older" patients is used. A 25% reduction in healing time, 3-4 days, is considered clinically significant. Assuming a standard deviation of about 3 days after adjusting for age, cause, and extent of burn, a total of 100 patients provides at least 95% power for one-tail t tests at the 0.05 α level comparing the treatment and control groups (including adjustments for multiple comparisons).

Criteria for Admission to the Study. Patients admitted to the US Army Institute of Surgical Research are offered the opportunity to participate in this study.

Patient Inclusion. Patients meeting the following criteria are considered for entry into the study:

1. Male or female patients > 18 and < 80 yr of age. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr of age and the lack of menstrual periods for at least 1 yr), or have a negative pregnancy test prior to initiation into the study.

2. Patients with flame or scald burns requiring a skin graft from the anterior upper thigh, buttock, or lateral upper arm. This site is the "test wound" and is evaluated for time of healing.

3. Patients with burns < LD 75, the size of burn at which 75% of the patients die at any particular age, using the US Army Institute of Surgical Research probit analysis (15).

4. Patients who have undergone successful resuscitation without major complication.

5. Patients who are able to take a minimum of 80% of maintenance energy, protein, and other nutrient requirements by the enteral or parenteral route.

6. Patients with inhalation injuries are eligible and may require mechanical ventilation, but a satisfactory PO_2 and PCO_2 will be required on < 60% oxygen.

7. Patients with a prehospitalization weight between 80 and 140% normal body weight as determined from standard tables for age and sex (Desirable Weight Tables, Metropolitan Life Insurance Company, 1959).

8. Patients with a single uncomplicated fracture of a long-bone are eligible.

Exclusion Criteria. Patients with the following characteristics are excluded from the study:

1. Patients < *18 or > 80 years of age.*

2. Patients who are pregnant or nursing.

3. Patients without flame or scald burns requiring a skin graft from the anterior upper thigh, buttock, or lateral upper arm.

4. Patients with burns > LD 75, the size of burn at which 75% of the patients die at any particular age, using the US Army Institute of Surgical Research probit analysis (15).

5. Patients who have had complications undergoing resuscitation.

6. Patients who are not able to take a minimum of 80% of maintenance energy, protein, and other nutrient requirements by the enteral or parenteral route.

7. Patients with inhalation injuries on mechanical ventilation with unsatisfactory PO_2 and PCO_2 or on > 60% oxygen.

8. Patients with a prehospitalization weight < 80 and > 140% normal body weight as determined from standard tables for age and sex (Desirable Weight Tables, Metropolitan Life Insurance Company, 1959).

9. Patients with associated head injuries which require specific therapy.

10. Patients with associated injuries to the chest or abdomen which require surgery or tube drainage.

11. Patients with multiple fractures.

12. Patients with a history of cancer within 5 yr or active neoplasia.

13. Patients with insulin-dependent diabetes mellitus.

14. Patients with renal failure (creatinine > 1.5 mg/dl).

15. Patients with hepatic disease (bilirubin > 3.0 mg/dl).

16. Patients with a past history of chronic infection such as AIDS or tuberculosis.

17. Patients with uncompensated congestive heart failure.

18. Patients with other chronic illnesses such as arthritis, cirrhosis, hyperlipidemia, or autoimmune disease requiring drug therapy.

19. Patients requiring chronic glucocorticoid or nonsteroidal anti-inflammatory drugs.

20. Patients with an established clinically significant nonburn wound-related infection.

21. Patients receiving any other experimental drug therapy within 2 months of the study.

Medication, Dose, and Administration. Recombinant human growth hormone is supplied for this study as sterile, lyophilized powders in vials containing 5 mg growth hormone. The placebo consists of excipient which is identical in appearance to the test drug. Each morning (beginning on the day of the first graft operation), patients receive either 5 or 10 mg recombinant human growth hormone or placebo by subcutaneous injection. Treatment continues for the duration of hospitalization. Other medications are administered as needed, including histamine antagonist, insulin, antihypertensive, cardiac, pain, and sleeping medications.

Wound Care. Graft donor sites evaluated as part of this study are on the anterior upper thigh, buttock, or upper arm.

These are the usual and preferred sites for taking skin for grafting. Skin is taken from the site with a dermatome set at 10/1000-in in thickness and full width. Two designated dermatomes are used exclusively for patients enrolled in this study. In addition, skin from the donor site is harvested by only two investigators. Only the first harvest of a donor site is used for this study. Fine-mesh gauze is applied to all donor sites. Bed cradles are used to assure that bed sheets do not displace the fine-mesh gauze and the patient is positioned so that the donor site is exposed to the air.

Laboratory Studies. Laboratory studies (see Table 1 for the study plan flow chart) are performed weekly and include complete blood count, serum chemistries, i.e., glucose, electrolytes, and liver and renal function tests, acute-phase proteins, i.e., transferrin and retinol-binding protein, urinalysis, free thyroxine, growth hormone antibodies (baseline and last day of study only), insulin levels (for patients not on exogenous insulin), and pharmacokinetics. Hematology, serum chemistries, thyroid function tests, and urinalysis are determined for all patients by Smith Kline Bioscience Laboratories and transferrin and retinol-binding protein by the Laboratory for Surgery Metabolism and Nutrition.

Following the tenth injection, a full 24-h pattern of endogenous and exogenous recombinant growth hormone levels are measured. A 3-ml sample is drawn into a standard tube without anticoagulants (red top) every 4 h and centrifuged, with samplings scheduled to include a sample drawn approximately 2 h after the onset of sleep when the usual normal surge of growth hormone occurs. Samples frozen on dry ice are then shipped to Genentech, Inc., via Federal Express as soon as possible after the sample is drawn.

Somatomedin-C, one measure of the index of activity of the recombinant growth hormone, is measured at each blood draw. A 2-ml blood sample is drawn into a standard tube containing EDTA (purple top) and centrifuged. The plasma is then removed and immediately frozen. Samples frozen on dry ice are shipped to Genentech, Inc., via Federal Express as soon as possible after the sample is drawn.

Physical Examinations. Physical examinations are performed daily and include weight, concomitant medications, vital signs, and any adverse events.

Nutrition. Near constant nutritional intake is provided beginning on the first postoperative day. This provides at least 80% of energy and protein requirements (calculated by standard formulae). The nutrients are provided in the same relative proportion throughout the study, with protein accounting for 15-25% total energy, carbohydrate providing

TABLE 1. Study Plan Flow Chart

	Baseline	Daily	Weekly
Physical examination*	X	X	
Burn evaluation	X		X
Vital signs	X	X	
Weight	X	X	
Concomitant medications	X	X	
Nutritional intake	X	X	
Complete blood count	X		X
Serum chemistries	X		X
Transferrin	X		X
Retinol-binding protein	X		X
Urinalysis	X		X
Free thyroxine	X		X
Somatomedin-C	X		X
Human growth hormone antibody test	X		X
Study medication		X	
Adverse events		X	

*Includes graft site evaluation with photographs beginning the third postoperative day.

50-80%, and fat providing 3-40%. Additional calories and protein can be provided, but they are not more than 25% of the estimated total requirements. Carbohydrate intake does not exceed 6 mg/kg/min (about 600 g/day for the usual patient). Nutrients are provided by the enteral or enteral-parenteral routes throughout the study and the route is altered according to the clinical course of the patient. Intake is monitored by the hospital dietitian and nutrition is supervised by a single nutritionist.

End Points.

Donor sites. On the third postoperative day, the wound is examined by a trained evaluator. The donor site wound is measured and photographed using a standard camera and distance. Using sterile technique, each of the four corners of the fine-mesh gauze is gently lifted to determine if the dressing is adherent to the underlying skin. This is done using sterile forceps and minimal tension. At the end of the examination, the unattached fine-mesh gauze is trimmed away with scissors and the dressing rephotographed. This examination procedure is performed each day until the fine-mesh gauze is completely removed. Complete removal indicates complete wound healing. Using the measurements and photographs, the fraction of the wound covered with fine-mesh gauze (unhealed) is plotted as a function of time. The time to 90% wound closure is compared between treatment and control groups.

Primary Burn. The extent of the burn is charted on graphs developed by the National Burn Information Exchange (16). Second and 3° burn areas are noted separately. The total area involved is calculated at the time of the first graft and weekly thereafter. The fraction of unhealed 2° and 3° sites are plotted as a function of time for the three treatment groups.

Length of Hospitalization. Length of hospitalization is defined as the time from admission until hospital discharge.

Nutrition. Nutritional intake is determined by daily calorie counts in patients who are eating spontaneously and/or from volume of parenteral nutrients.

Infection Rates/Mortality. Infection rates and mortality are compared among the three treatment groups.

Statistical Analyses. Mortality (survival time) and healing time of the donor graft site are the primary end points. Differences between treatment and control groups will be assessed with respect to mortality using Cox model regression survival analysis, with age, cause of burn, and extent of burn as covariates. Differences between treatment and control groups with respect to healing time of the donor graft site for patients who do not die will also be evaluated using analysis of covariance, with age, cause of burn, and extent of burn as covariates.

Healing time of the primary burn site and length of hospitalization are secondary end points, the analysis of which will be similar to that for healing time of the donor graft site. Nutritional intake and infection rates will also be secondary end points for which appropriate comparisons will be made between treatment and control groups.

Adverse events are tabulated and appropriate comparisons will be made between treatment and control groups. Laboratory safety data are tabulated and values outside normal limits identified.

RESULTS

This project was approved by the USAISR Research Council, US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the third quarter of fiscal year 1988. Three patients have been enrolled in the study since mid-May. No adverse reactions or side effects were noted.

DISCUSSION

When 100 patients have been enrolled in the study, the code will be broken and data analyzed for the effect of recombinant human growth hormone on wound healing in thermally injured patients.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA312333	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	CA	166
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code)
(U) High Frequency Ventilation in Patients with Inhalation Injury

12. SUBJECT AREAS
06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 03	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY *Basil A. Pruitt*
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.1	6
89	0.1	8

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): MC MANUS, W F
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22. KEYWORDS (Precede EACH with Security Classification Code) (U) High Frequency Ventilation; (U) Inhalation Injury; (U) Thermal Injury; (U) Pneumonia; (U) Morbidity;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Mortality; (U) Volunteers: (U) Adults; (U) RAII

23. (U) To evaluate the use of high frequency ventilation in the treatment of respiratory failure in patients with inhalation injury with respect to incidence of pneumonia, time of initial onset and duration of pneumonitis, total duration of ventilatory support, and the age and burn size-adjusted mortality. A literature search was performed and indicated no duplication of effort.

24. (U) Initial high frequency ventilation support will be established by adjustment of the driving pressure, rate, and inspiratory-expiratory ratio. A chest roentgenogram will be obtained and sputum or endotracheal secretions will be examined by culture and Gram stain. It will then be determined how high frequency ventilation affects outcome after inhalation injury with respect to several well defined factors as compared to an historical cohort using conventional ventilation. The incidence of pneumonia will be related to burn size by appropriate burn-size stratification within the study group.

25. (U) 8710 - 8809. Fifteen patients have been entered into the study, 14 during this reporting period. After the entry of 50 patients, the effects of high frequency ventilation on the outcome after inhalation injury with respect to several well-defined factors, to include mortality and pulmonary morbidity, will be compared to an historical cohort.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: High Frequency Ventilation in Patients with Inhalation Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William G. Cioffi, Jr., MD, Captain, MC
Theresa A. Graves, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

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William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Inhalation injury complicated by bacterial pneumonia is now one of the primary causes of morbidity and mortality in patients with thermal injury. We have investigated the use of high frequency percussive ventilation (HFPV) as a means of ventilatory support for these patients. We propose that high frequency ventilation (HFV) may decrease the incidence of pulmonary infection following inhalation injury and decrease the incidence of iatrogenic barotrauma caused by conventional ventilation.

HFV was instituted initially as salvage therapy in a group of 5 patients. In each case, normocapnia, or arterial PO_2 saturation of > 90% on a FIO_2 of 60% or less, was achieved with HFV but not with conventional ventilation. A second group of 10 patients was prospectively entered into this study on the use of HFPV in patients with inhalation injury. One patient was removed from the study and one patient was unable to be ventilated due to severely noncompliant lungs. Eight patients with a mean age of 29 yr and a mean burn size of 38% of the total body surface area completed the study. All patients survived; 2 patients developed penumonia and 1 patient developed subcutaneous emphysema. These results suggest that HFPV is effective in the treatment of patients with severe inhalation injury.

HIGH FREQUENCY VENTILATION IN PATIENTS WITH INHALATION INJURY

Inhalation injury continues to be a significant occurrence in the thermally injured patient (1). With the advent of topical chemotherapy, burn wound sepsis has been greatly reduced. However, this has been replaced by bacterial pneumonia as the primary cause of infectious morbidity and mortality stemming from the initial burn injury. Inhalation injury has been shown to predispose to the development of bacterial pneumonia, with 38% of patients with inhalation injury developing pneumonia as compared to 8.8% of those patients without inhalation injury (2). Furthermore, in this most recent analysis, it has been shown that inhalation injury complicated by pneumonia results in a maximum 60% increase in mortality as opposed to a maximum 20% increase in mortality when inhalation injury alone is added to burn mortality.

The pathophysiology of inhalation injury is the subject of active research. Extensive tracheobronchial injury is known to result from the initial insult, with sloughing of respiratory mucosa and resultant impairment of the normal mucociliary clearance mechanism of the tracheobronchial tree. In addition, disruption of the alveolar capillary membrane results in a protein-rich plasma exudate in the terminal airway which, coupled with impaired transport, serves as a set up for bacterial overgrowth and consolidated pneumonia. Injury to Type II pneumocytes with impairment of surfactant production contributes significantly to the pathologic process.

Current treatment modalities for progressive respiratory insufficiency resulting from inhalation injury involve support with conventional volume-cycled positive pressure ventilators to promote alveolar ventilation. Supplemental oxygen and standard methods of tracheobronchial toilet are also employed. Additionally, systemic antimicrobial treatment, as dictated by results of cultures and sputum microbiology, are administered to patients who develop clinical pneumonia.

Unfortunately, the outcome observed with conventional mechanical ventilatory management is usually one of ever worsening pneumonia. A documented spiral of pulmonary dysfunction ensues with progressive pulmonary shunting and carbon dioxide retention, necessitating ever increasing minute ventilation. Difficulty in arterial oxygenation necessitates administration of toxic levels of oxygen. Decreasing pulmonary compliance results in excessive peak airway pressures to maintain alveolar ventilation. This adds significant barotrauma which may cause further airway damage.

A means of pulmonary support which would more effectively aid in the recovery from the initial insult would be of great clinical benefit. An alternative to the current method of

ventilatory support which has proven to be of some benefit in other settings requiring artificial ventilation has been the high frequency ventilator (3-4). This technique is approved for use where large pulmonary air leaks preclude adequate ventilation with conventional techniques, e.g., bronchopleural fistulas (5). This method has also been shown to be efficacious during operative procedures where a quiet operative field is of benefit (6), to prevent deoxygenation during tracheal suctioning (7), in the treatment of aspiration (8), while performing endotracheal tube changes (9), and during laryngoscopy (10).

High frequency ventilation (HFV) has been reported to be effective in a number of clinical and laboratory trials (11-12). This technique employs tidal volumes less than physiologic dead space (1-5 ml/kg) at rapid respiratory rates (1-15 hertz) as compared to conventional ventilation which employes high tidal volumes at low frequencies. The utility of this method of ventilatory support is still being defined, but several potential advantages over conventional ventilation exist in the burn patient. For a given level of PEEP, peak airway pressure is significantly less for HFV than conventional ventilation, thus conceivably reducing the risk of barotrauma (2,13-15). Also, smaller fluctuations in peak airway pressure and mean airway pressure associated with HFV may have less effect on hemodynamic function. Oxygenation is also improved, resulting in lower levels of inspired oxygen, presumably secondary to increased diffusion. Additionally, recruitment of collapsed alveoli is prompted by the oscillatory nature of the inspired breaths in association with the use of PEEP. Limited clinical experience with HFV in burn patients has shown that clearance of secretions from the tracheobronchial tree is dramatically increased when a patient is converted from conventional ventilation to HFV. Increased turbulence of flow has been postulated as the responsible mechanism. Conventional ventilation, on the other hand, delivering large tidal volumes at high pressures only drives these secretions farther into lower airways, exacerbating the pneumatic process. The ability to ventilate burned patients with inhalation injury resulting in lower peak and mean airway levels, lower levels of PEEP, and lower FIO_2 while at the same time enhancing clearance of secretions and improving recruitment of collapsed alveoli should at the very least limit the compounding damage normally superimposed by conventional ventilation on the initial insult. Whether this will allow the lung to recover more quickly and reduce the incidence of pneumonic complications is unknown.

The objective of this study is to evaluate the effect of HFV in the treatment of respiratory failure in patients with inhalation injury with respect to incidence of pneumonia, time of initial onset and duration of pneumonitis, total duration of ventilatory support, and the age and burn-size adjusted mortality.

MATERIALS AND METHODS

Patient Population. Fifteen patients admitted to the Institute between December 1986 and February 1988 formed the basis for these results. Inhalation injury was confirmed in each patient by bronchoscopy and xenon scan. The presence of erythema and ulcerations was used to define moderate to severe inhalation injury. Patients with significanty positive xenon scans but normal bronchoscopy were defined as mild inhalation injury. This classification schema was based upon our previously published review (2).

Group I consisted of 5 patients in whom HFV was initiated as salvage therapy. Each patient failed conventional, mechanical ventilatory support using either a Bear II or Seiman's 900C ventilator. In each case, the ability to maintain normocapnia or an arterial oxygen saturation > 90% on a level of inspired oxygen of < 70% was impossible with conventional ventilation.

Group II consisted of the first 10 patients enrolled in this study. Each patient met standard criteria for the need of mechanical ventilation, i.e., respiratory rate > 30/min, PaO_2 < 60 on a FIO_2 > 40%, minute ventilation > 20 L/min, PCO_2 > 45 or PCO_2 < 45 but progressively increasing, severe laryngeal edema, and severe facial burns. Mechanical ventilation was instituted with the high frequency ventilator within 24 h of intubation.

Criteria for Admission to the Study. Patients admitted to the US Army Institute of Surgical Research with evidence of inhalation injury were offered the opportunity to participate in this study.

Patient Inclusion. Patients meeting the following criteria were considered for entry into the study:

1. Male or female patients > 18 yr of age. Female patients were previously surgically sterilized or postmenopausal (> 45 yr of age and the lack of menstrual periods for at least 1 yr) or had a negative pregnancy test prior to initiation into the study.

2. Patients admitted to the Institute within 48 h of the burn injury. Patients aeromedically transferred who were previously on conventional ventilatory support were eligible for entry if the total duration of ventilatory support was < 48 h.

3. Patients with a history of inhalation injury confirmed by xenon lung scan or bronchoscopy. All patients with a positive xenon lung scan had bronchoscopy.

4. Patients with a clinical need for ventilatory support as deemed appropriate by current clinical practice.

Patient Exclusion. Patients with the following characteristics were excluded from the study:

1. Any patient < 18 yr of age.

2. Any patient admitted to the Institute > 48 h postinjury.

3. Any patient without confirmed inhalation injury.

4. Any pregnant patient.

5. Any patient being treated with antibiotics for diagnosed pneumonia. Administration of prophylactic antibiotics did not exclude entry into the study.

Procedure Prior to Patient Entry. Any patient deemed eligible based on the above criteria had the following documented prior to initiation of the study:

1. A history and physical examination.

2. A chest roentgenogram within 24 h.

3. Arterial blood gases within 6 h.

4. Sputum culture and microscopic examination within 24 h.

5. Informed consent.

High Frequency Percussive Ventilator. HFPV was delivered by a high frequency pulse generator (Bird Space Technologies, Percussionaire Corporation, Sand Point ID). Gas from the high frequency pulse generator was delivered through a nongated sliding venturi connected to a standard endotracheal tube. The venturi entrained humidified gas from a fresh bias flow provided from the ventilator. The system combined a series of high frequency sub-dead space volume breaths with a variable I:E ratio. Initially, the I:E ratio for the subtidal volume breaths was 1:1. Periodic interruption of high frequency pulsatile flow was programmed to allow return of airway pressure to baseline CPAP. The ratio of the duration of the percussive phase to the duration of baseline CPAP was adjusted as a means of manipulating oxygenation and CO_2 elimination. Additionally, peak airway pressure could be adjusted to maintain adequate CO_2 elimination. The frequency of the sub-dead space breaths was maintained between 200-600 breaths/min. FIO_2 and PEEP were adjusted to maintain an O_2 saturation > 90%.[2]

Study Design. In Group I, each patient's peak airway pressure, FIO_2, PEEP, PCO_2, and PO_2 were compared before and after initiation of HFV. In Group II, the incidence of clinically significant barotrauma, the incidence of pneumonia, and ultimate outcome were recorded.

The recent experience with inhalation injury in 373 patients during a 5-yr period at this Institute was reviewed (3). The specific contribution of inhalation injury and pneumonia to age and burn-size dependent patient mortality was defined in this population using multiple logistic regression analysis. In addition, postburn day of diagnosis of pneumonia in patients with inhalation injury and those without inhalation injury was defined. In the present study, we determined how HFV affects outcome after inhalation injury with respect to these well defined factors in comparison to the historical cohort using conventional ventilation. The incidence of pneumonia was related to burn size by appropriate burn size stratification within the study group.

RESULTS

In Group I, 5 patients were treated with HFPV as salvage therapy. Patient characteristics for this group are listed in Table 1. Blood gas data just prior to and after 6 h of HFPV are shown in Table 2. Prior to its institution, each patient demonstrated either progressive hypoxemia or CO_2 retention and were unresponsive to alterations in FIO_2, PEEP, I:E ratio, and minute ventilation. Each patient underwent multiple manipulations of mechanical ventilatory support, dependent upon whether hypoxemia or CO_2 retention was present. Attempts to improve oxygenation included increased levels of PEEP (up to 25 cmH_2O), inversed I:E ratios, and increases in FIO_2. Attempts to improve CO_2 clearance included increases in respiratory rate up to 40 breaths/min, alterations in peak flow, and increases in tidal volume (up to 15-18 ml/kg). Despite considerable blood gas improvement following initiation of HFPV, each patient eventually died.

In Group II, 10 patients were entered into a prospective study of the use of HFPV in patients with inhalation injury from March 1987 to February 1988. Inhalation injury was diagnosed by bronchoscopy in each patient and was considered moderate to severe in nature. One patient was removed from the study upon request of the attending physician and one was switched back to conventional mechanical ventilation due to inability of the high frequency ventilator to deliver adequate inspiratory pressures (>120 cmH_2O). Seven patients completed the study and one patient was still participating. Patient data are presented in Table 3. The mean age of these 8 patients was 29 yr (range 19-60) with the extent of burn varying from 10-65% of the total body surface area ($\bar{x}$=38). The average number of ventilator days was 11 (range 4-30). The

TABLE 1. Characteristics of Patients in Group I

Patient Number	Age	Sex	Burn Size* (%)	Treatment Initiated (PBD)	Complications
1	4	M	44	18	Pneumonia
2	63	M	68	16	Pneumonia, Sepsis
3	2	M	43	1	-
4	1	F	59	11	Pneumonia
5	5	M	75	12	Pneumonia

*Percentage of total body surface area.
PBD indicates postburn day.

TABLE 3. Blood Gas Data for Group I (Conventional Ventilation/HFV)*

Patient Number	FIO_2	PIP (cmH_2O)	PEEP (cmH_2O)	P_aO_2/FIO_2	P_aO_2	P_aCO_2
1	1.0/0.5	50/38	7/5	63/172	63/86	57/37
	1.0/0.45	50/50	10/10	103/228	103/103	158/70
2	1.0/0.5	65/50	10/2	149/236	149/118	46/48
3	1.0/0.4	40/20	10/5	60/352	60/141	51/32
4	0.7/0.4	70/70	10/10	140/325	98/130	77/40
5	1.0/0.6	75/65	20/3	36/175	36/105	55/27

*Represents best obtainable ventilator settings and blood gases for each patient.

only clinically evident barotrauma was subcutaneous emphysema in one patient. Two patients developed pneumonia. All patients survived.

DISCUSSION

When inhalation injury accompanies cutaneous burns, mortality is disproportionately higher than that predicted by the extent of cutaneous injury alone (16). This synergistic

TABLE 3. Characteristics of Patients in Group II

Patient Number	Age	Sex	Burn Size* (%)	Predicted Mortality (%)	Ventilator Days	Complications
1	27	F	56	65	17	Pneumonia
2	35	M	37	35	4	-
3	20	M	51	55	5	-
4	20	M	38	35	12	-
5	31	F	31	25	6	-
6	19	M	17	20	7	Barotrauma
7	19	M	10	20	7	-
8	60	M	65	79	30+	Pneumonia

effect is most apparent in patients with burns associated with a 40-60% mortality. Inhalation injury alone results in a maximum 20% increase in mortality. The incidence of bacterial pneumonia is greatly increased by the presence of inhalation injury, with 38% of patients with inhalation injury developing pneumonia as compared to 8.8% of burn patients without inhalation injury. When inhalation injury was moderate to severe, i.e., diagnosed by bronchoscopy, 48% developed pneumonia. The addition of pneumonia to inhalation injury results in a maximum 60% increase in mortality for this group of patients (2).

The pathophysiology of inhalation injury is complex and not fully understood. Extensive tracheobronchial injury may result in sloughing of the respiratory tract mucosa and impairment of the normal mucocilliary clearance mechanism. Slough of the mucosa results in cast formation which obstructs moderate-sized airways, leading to distal atelectasis, or a ball-valve effect, leading to distal air trapping and increased barotrauma. Disruption of endothelial and epithelial integrity permits exudation of protein-rich plasma into terminal airways which serves as media for bacterial overgrowth and subsequent development of pneumonia. Injury to Type II pneumocytes impairs surfactant production and contributes significantly to the pathologic process. The parenchymal changes are associated with hypoxemia and hypercapnia due to the shift of the V/Q relationship to the left, i.e., an increase in lung segments with V/Q ratios > 0 but < 1. This change in V/Q has been adequately documented in animal models of inhalation injury (17).

The goal of any new treatment regimen for inhalation injury should be the reversal of these pathophysiologic changes. A second and equally important goal is that the treatment causes no further injury. For patients with moderate to severe injury who require mechanical ventilation, current conventional ventilators do not accomplish either of these goals. Clearance of secretions is not enhanced, and the complications of high inflation pressures and high FIO_2 compound the existing injury. The goal of ventilator therapy should be adequate oxygenation on a nontoxic FIO_2 and CO_2 clearance at the lowest possible inspiratory pressures, with maintenance of functional residual capacity above closing volume. Better clearance of secretions and a shift of the V/Q mismatch back to the right are also desirable. Various reports have concluded that high frequency ventilation may accomplish all of these goals (13,18-23).

The major characteristics of HFV include a ventilatory frequency > 60 breaths/min, tidal volumes of less than dead space, lower peak airway pressures than conventional ventilation, positive endotracheal pressures throughout the ventilatory cycle, lower transpulmonary pressures than in conventional ventilation, increased FRC, possibly less

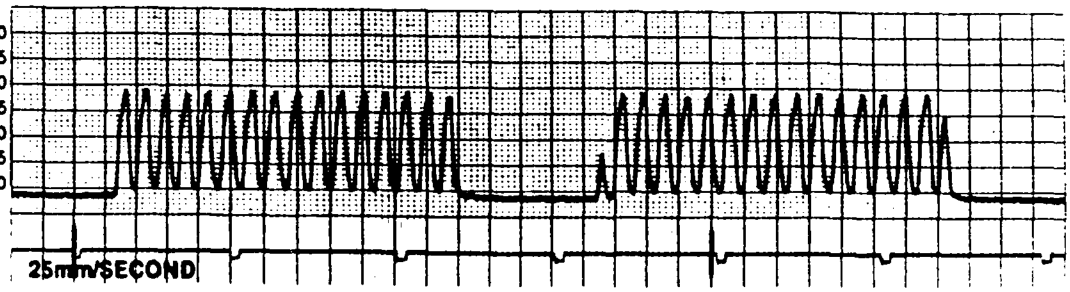
circulatory interference than with conventional ventilation, and more efficient pulmonary gas distribution than with conventional ventilation (20). These characteristics of HFV should make it the ideal form of ventilatory support for patients with significant inhalation injury but it should be noted that each of these reported advantages has been refuted in various reports (24-26).

The safety of HFV has been documented in several reports. Its use in postoperative general surgery and cardiac surgery patients has demonstrated no untoward hemodynamic effects (27). Peak airway pressures and mean airway pressures are reduced and several studies have demonstrated decreased pulmonary shunt flow with the use of this type of ventilatory support. Two recent reports have documented the effectiveness of short-term salvage use of HFV in patients with ARDS (19,20). Oxygenation was improved and adequate CO_2 clearance was maintained in each patient.

Carlon et al (11) reported a series of 309 patients who were randomized to high frequency jet ventilation or conventional ventilation, respectively. Mortality from underlying disease was quite high in that study; however, the pulmonary dysfunction was not severe. Mean ventilator time was 2.5 days. Peak airway pressures were lower in the HFV group as compared to the conventional ventilation group. There was a 4% incidence of barotrauma in each gorup. Overall outcome did not differ between the two groups. Standard high frequency jet ventilation at a rate of 100 breaths/min and an I:E ratio of 1:2 was used in each patient. The only manipulated variable was driving pressure. In an attempt to standardize the ventilatory support for each patient, the versatility of high frequency jet ventilation was not utilized, i.e., frequency and I:E ratios were not altered to obtain the best possible result.

HFPV is an exceptionally versatile form of HFV in which high frequency subtidal breaths are superimposed upon conventional ventilation (Fig 1). This ventilator combines the entrainment mechanism of high frequency jet ventilation with the ability to manipulate airway pressure in a phasic manner.

In this study, we have demonstrated the superiority of HFPV as compared to conventional ventilation in maintaining adequate CO_2 clearance and oxygenation in a critically ill group of burn patients with severe respiratory insufficiency (Group I). For this group of patients, recommendations of optimum ventilatory settings cannot be made. For both conventional and high frequency ventilators, all ventilator settings were manipulated to achieve the best possible oxygenation and CO_2 clearance at the lowest possible peak inspiratory pressures and PEEP values. In this small group of severely ill patients, mortality from multiple organ failure was not altered, even though ventilation was improved. At the time HFPV was instituted in each patient



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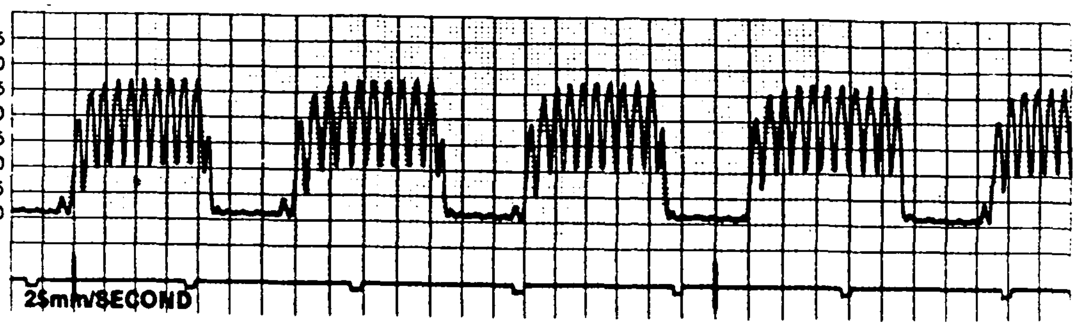

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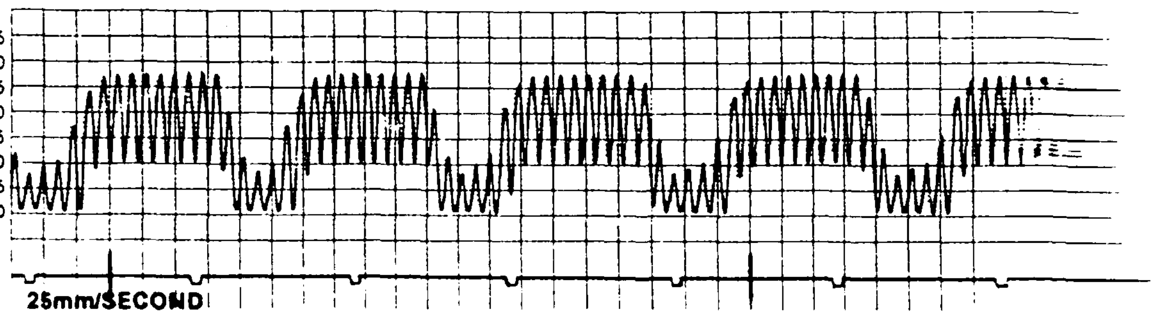

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FIGURE 1. Representative wave form tracings from proximal airway using HFPV. **A**, Standard wave form with periodic interruption of pulsatile flow. Note reverse I:E ratio. **B**, Standard wave form with higher inspiratory pressures and increased convective gas flow. **C**, Standard wave form with institution of oscillatory PEEP during period of interruption of pulsatile flow.

as salvage therapy, at least two other systems besides pulmonary function had already failed.

The results from the prospective use of HFV in Group II are favorable. The degree of pulmonary insult was moderate to severe in all patients as assessed bronchoscopically. Using data collected from an historical cohort treated at this Institute between 1980-5, one would predict an approximate 40% mortality for patients in Group II (2). Additionally, approximately one-half of the patients should have developed pneumonia. In actuality, all patients survived and only two developed pneumonia during their hospital course. Although no firm conclusions can be drawn because of the small number of patients in Group II, a favorable trend is noted. To determine whether HFV significantly improves survival, approximately 50 study patients will be needed.

The results of this study support but do not confirm the opinion that HFV is effective in the treatment of patients with severe ARDS. Although it is unlikely that HFV represents a panacea for all forms of respiratory failure, the results for Group II suggest that HFV will be useful in the treatment of patients with severe inhalation injury. Group II patients were different than those with severe ARDS in that the insult was acute in nature, and given the proper circumstances, should heal in 10-21 days. If during this period of spontaneous airway repair one can provide adequate ventilatory support, not increase the risk of barotrauma, and at the same time facilitate clearance of airway secretions and debris resulting from the injury, one may be able to decrease the morbidity and mortality associated with inhalation injury.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA313318	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	EC	167
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Quantification of Dynamic Splint Forces on Metacarpophalangeal Function Recovery

12. SUBJECT AREAS

06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 10	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

a. DATE EFFECTIVE APPROVED BY: [signature]

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.0	45
89	1.0	25

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: LUSTER, S H
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-5957
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): PATTERSON, P E
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available): CIOFFI, W G

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Metacarpophalangeal Joint Stiffness; (U) Dynamic Flexion Splint; (U) Force Quantification; (U) Volunteers:

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Adults; (U) RAII.

23. (U) To develop instrumentation to quantify initial stiffness in metacarpophalangeal joints and, using this instrumentation, to compare the effects of different splinting regimens in the rehabilitation of burned hands. Data from this study will be used to modify and improve hand rehabilitation of injured soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) An electronic joint stiffness monitoring device will be designed and tested. In addition, two methods of metacarpophalangeal joint flexion splinting be developed. Selected patients requiring hand rehabilitation will be measured with the device and then be placed in one of four treatment groups with serial remeasurements to determine treatment effectiveness.

25. (U) 8710 - 8809. A prototype splint monitoring device was designed, fabricated, and tested. On the basis of four case studies, it was determined that the stiffness of a target joint could be reliably quantified using the splint monitoring device. Plans have been made to miniaturize the device for ease of data gathering and begin the comparative study of metacarpophalangeal flexion splinting.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * USGPO 1986-491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: Quantification of Dynamic Splint Forces on Metacarpophalangeal Function Recovery

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Stephen H. Luster, OTR, Major, SP
Patrick E. Patterson, PhD*
William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

**Iowa State University*
of Science and Technology
Ames, Iowa 50010

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: Quantification of Dynamic Splint Forces on Metacarpophalangeal Function Recovery

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: Stephen H. Luster, OTR, Major, SP
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William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Hand rehabilitation following burn injury is frequently complicated by joint stiffness at the metacarpophalangeal (MCP) joints and is treated with rubber band-powered flexion traction splints. A device to quantify finger joint stiffness while mimicking the forces applied by a splint has been developed and used to investigate the effects of splinting. The device consists of an electric motor/hydraulic piston system mounted on a dorsal wrist splint which is controlled by a computer. As the device moves a finger through its range-of-motion, the computer calculates the resistive forces and identifies a stiffness value at an angular position. In the pilot study phase of this project, 4 subjects with a total of 20 stiff MCP joints were evaluated. Each subject's hand was randomly assigned to a 1- or 2-h treatment group for 3 days of standard MCP flexion traction splinting. Based on initial measuresmsnts of joint stiffness, all joints were assigned to a low or high stiffness group. Between the first and third treatment days, there was a mean increase of 34% in the angle at which maximum resistance (stiffness) was encountered. There were statistically significant differences between the low and high stiffness groups in angular location of resistance ($P < 0.05$) and amount of resistance ($P < 0.0001$). There were no significant differences between the 1- and 2-h treatment groups. This initial trial suggests that finger stiffness can be quantified, traction splinting does alter joint stiffness, allowing increased motion, the amount of initial joint stiffness may be an indicator of treatment outcome, and increasing treatment time may not necessarily enhance outcome.

QUANTIFICATION OF DYNAMIC SPLINT FORCES ON METACARPOPHALANGEAL FUNCTION RECOVERY

Joint stiffness of the hand with resultant decrease in hand function is a frequent complication of burn injury. Prolonged edema and immobilization following skin grafting can result in loss of motion of all joints of the hand. While the primary concern in preservation of the deeply burned hand is protection of the extensor hood mechanism of the proximal interphalangeal joints (1), the metacarpophalangeal (MCP) joints must also be protected from the onset of stiffness. The MCP joints are critical joints in overall function of the hand. They are the "gate keepers" of hand function in that they position the distal phalanges to perform full opening or closing of the hand as well as all other manipulative motions. Loss of motion at these joints severely limits hand function. Following burn injury, the MCP joints are subject to the development of stiffness from shortening of the collateral ligaments and adhesions of the dorsal synovial pouch (2). Maintaining and, if necessary, regaining MCP joint motion is one of the primary goals of rehabilitation of the burned hand.

Dynamic or traction splinting is one of the modalities commonly used in hand burn rehabilitation (3-6). This type of splinting uses rubber bands to apply a low amplitude force to a joint for an extended period of time. Because human tissues are viscoelastic, they respond to the forces applied by a traction splint in specific ways (7). If force is applied to tissue for a short period of time, the tissue will display an elastic response. It will first be elongated by the force but then return to its initial shape when the force is released. If the force is of low magnitude and prolonged, the tissue will display a viscous or plastic response and remain elongated after removal of the force. This elongation is caused by cell proliferation in the stressed tissue (8).

While hand rehabilitation literature frequently cites the use of dynamic splints for treatment of MCP stiffness, there have been no studies documenting the quantification of joint stiffness or the specific amount of force to be applied during splint application. This lack of objective data on which to base splinting procedures has resulted in treatment protocols which are based on isolated clinical experience and anecdotal information. The development of a methodology to quantify joint stiffness may allow the establishment of scientifically based treatment protocols and result in increased splinting effectiveness and rehabilitation outcome.

The objectives of this study are to develop instrumentation and a methodology to quantify joint stiffness in the burned hand, and with this capability, assess the effects of different treatment protocols on MCP recovery. A pilot study using the

prototype device will be completed followed by a study of 100 burned hands treated within one of six treatment groups (2 splint types, 2 treatment durations, and 2 force levels).

MATERIALS AND METHODS

A prototype device capable of concurrently producing and measuring forces similar to those applied to a phalanx by a dynamic splint was designed and constructed by the Biomedical Engineering Department at Iowa State University (Ames IA). The device consists of a dorsal thermoplastic wrist control splint which supports an electromagnically controlled hydraulic piston liked to a finger "cradle", a single-board microcomputer configured to control the position and rate of movement of the piston, and a laptop computer which serves as a master controller as well as providing data logging and analysis functions. The system was configured to apply a torque at a constant 90° angle to the proximal phalanx and to match the reactive moment being generated by the involved joint. As the phalanx was moved through its range-of-motion, the resulting curve of reactive torques and their associated angular locations were logged. The first measurement logged was the angle at which initial resistance was encountered and was coded IANG. The computer then calculated the rate of change (torque/°) and located the point at which the largest change occurred. This point was coded as final angle (FANG) and, along with the resistive moment (RMO) at this point, was selected to characterize joint condition or stiffness. Due to limited availability of hand-burned patients at the time the prototype device was available for use, only 4 patients with a total of 20 stiff MCP joints participated in the initial trial of the device (see Table 1). Based on initial values of primary resistance, each joint was assigned to either a high or low stiffness group. The patients were then randomly assigned to one of two treatment groups. Group I received standard MCP joint dynamic flexion splinting with a ventral rubber band-powered splint for 1 h/day. Group II received the same treatment for 1 h twice a day. Each participant received 3 days of treatment, with primary resistance measures being taken before and after each session. A fourth measurement was taken on day 6 after 2 days of no splinting to additionally assess the effects of discontinuation of treatment. A two-way ANOVA of treatment days and the variables IANG, FANG, and RMO was conducted. In addition, a 2 (stiffness groups) X 2 (splinting protocols) X 4 (days) ANOVA with repeated measures on the last factor was performed.

RESULTS

There were statistically significant differences in the mean values of the variables IANG, FANG, and RMO between the first and third treatment days and between the first and sixth days (Fig 1). There was an 18% increase in IANG followed by a

TABLE 1. Subject Data

	SUBJECTS A	B	C	D
Age (Yr)	32	23	31	26
Sex (Male/Female)	M	M	M	M
Burn Size (%)	80.5	10.25	35	70
Type of Injury	Flame	Flame	Flame	Flame
Hand Dominance (Left/Right)	R	R	R	R
Postburn Day Treatment Initiated	35	72	147	137
Number of Digits Involved	8	2	3	7

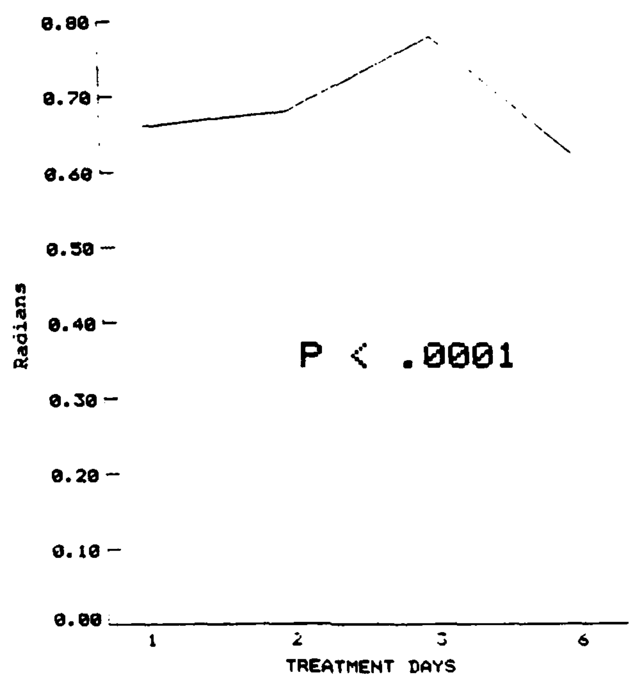

FIGURE 1A. Mean change in variable IANG.

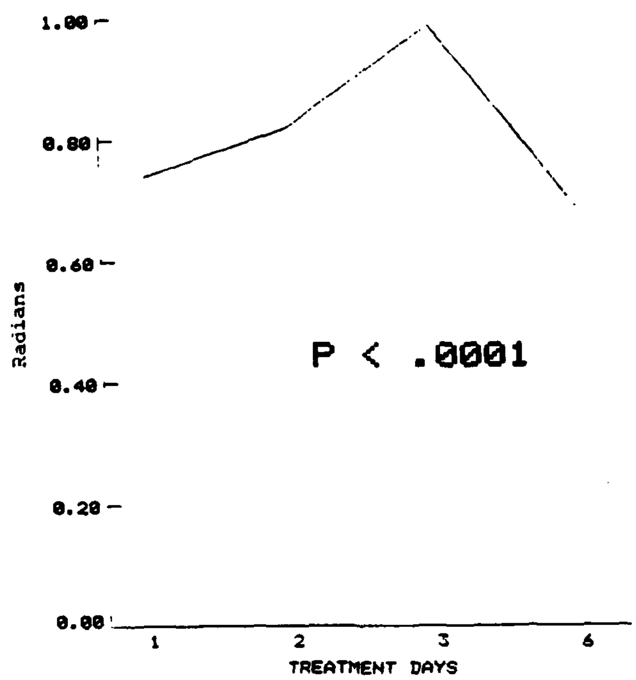

FIGURE 1B. Mean change in variable FANG.

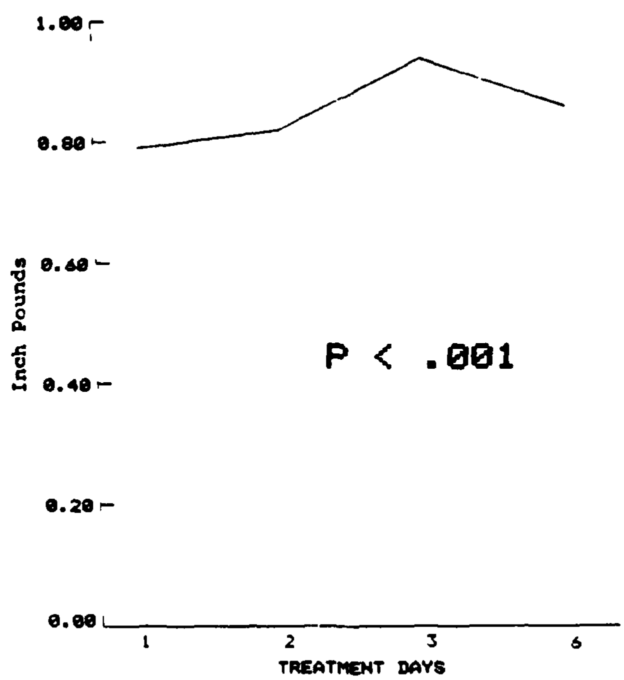

FIGURE 1C. Mean change in variable RMO.

6% decrease after 2 days of no splinting ($P < 0.001$). The FANG increased by 34%, then decreased by 6% ($P < 0.001$). Figure 2 illustrates the relationship of these three variables over the treatment period. It appeared that while the splinting resulted in increased joint angle, there was also an increase in the RMO on day 3 when the angle increased. The RMO decreased thereafter. A Duncan multiple-range ANOVA was used for the variables treatment days, angular position of initial resistance, RMO, stiffness groups, and length of treatment time. There were statistically significant differences between the low and high stiffness groups in angular position of resistance (FANG) ($P < 0.05$) and amount of resistance (RMO) ($P < 0.0001$). There were no significant differences between the 1- and 2-h treatment groups.

DISCUSSION

While this trial of the prototype splint monitoring device was limited in subject number and duration, the results demonstrated that it is possible to quantify stiffness in target joints of the burned hand. The data suggest that dynamic or traction flexion splinting of the MCP joints does decrease joint stiffness and increase motion over time. The two-day trend of increasing angle and resistance followed by continued increase in angle with decreased resistance may be an indicator of decreasing joint stiffness. The initial increase in the ratio of FANG to RMO followed by a decrease after treatment was discontinued suggests that treatment effects may only be temporary unless carried out for a longer period than 3 days. The significant changes in joint stiffness between the low and high stiffness of a joint may be an indicator of treatment outcome. While an optimum duration of treatment was not defined, the lack of significant change in joint stiffness as the result of treatment time does suggest that increased treatment time may not necessarily enhance outcome. Further study of normal hands and a larger sample of burned hands is indicated to determine the long-term relationship of IANG, FANG, and RMO with treatment type, duration, and frequency. When used in this pilot study, the prototype splint monitoring device was cumbersome, requiring two people for data collection. The device is currently being revised for ease of data collection and will be used in the continuation of this study.

PRESENTATIONS/PUBLICATIONS

None.

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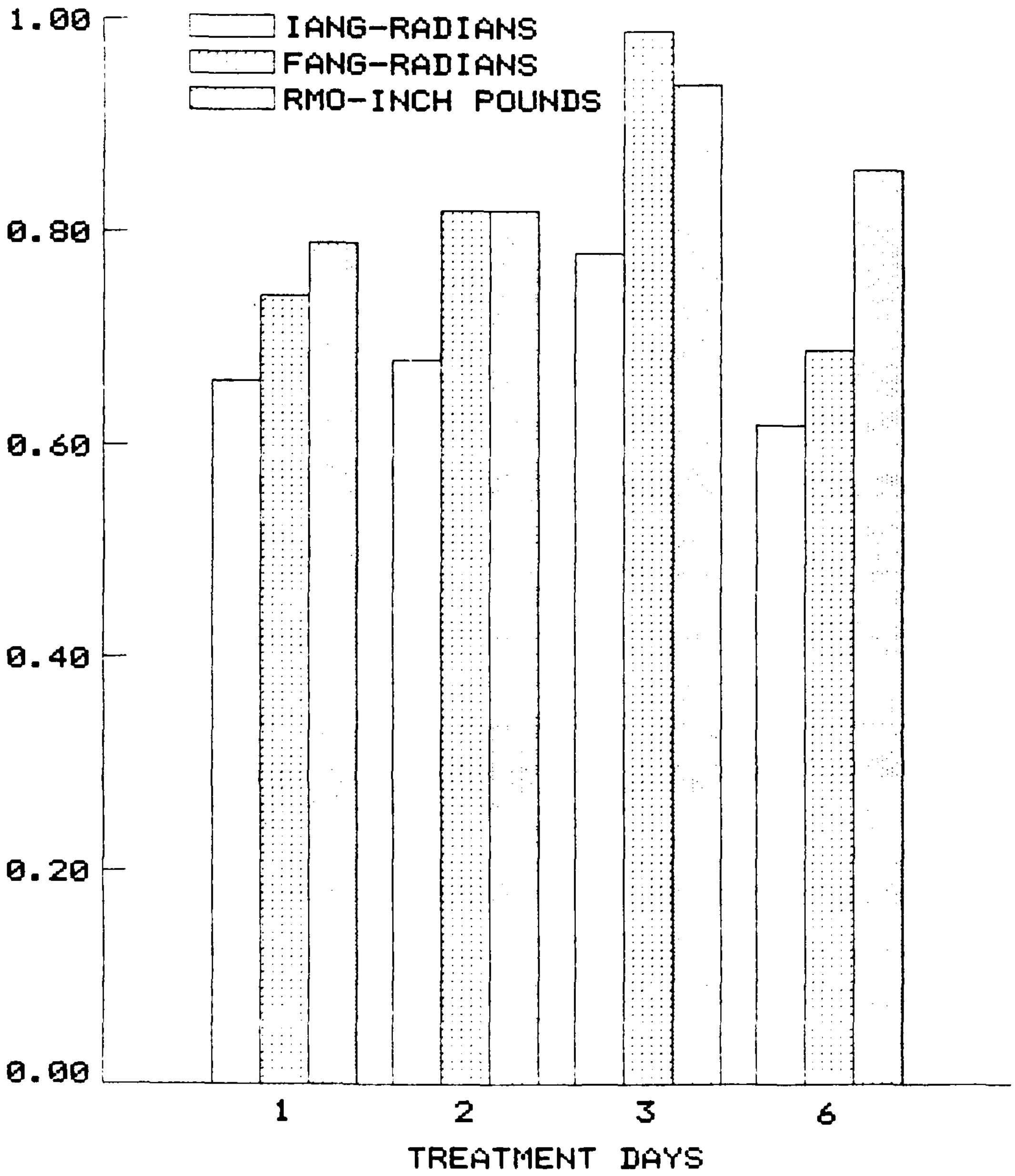

FIGURE 2. Mean changes in variables IANG, FANG, and RMO.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA314853	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
88 06 23	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S161787A874	F	169
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Phase II Study of Human Recombinant Granulocyte Macrophage Colony-Stimulating Factor in Patients with Thermal Injury

12. SUBJECT AREAS

06 05 Medicine and Medical Research 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
88 06	92 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

a. DATE EFFECTIVE APPROVED BY Basil A. Pruitt

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.3	5
89	0.8	32

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR CIOFFI, W G
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-4440
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) BURLESON, D G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) MC MANUS, W F

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns; (U) Immunosuppression; (U) Colony-Stimulating Factor; (U) Granulocytes; (U) Macrophages;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Lymphokines; (U) Pharmacology; (U) Volunteers: (U) Adults; (U) RAII

23. (U) Although early mortality from thermal injury has declined over the last several years, late mortality remains high, primarily due to infection. Despite other improvements, no significant impact has been made upon the defects in the immune system and their clinical consequences which contribute to mortality. Granulocyte macrophage colony-stimulating factor (GM-CSF) has the ability to stimulate production of granulocytes and also stimulate various functional activities of those cells. This study will determine the safety and activity of human recombinant GM-CSF in patients with thermal injury. A literature search was performed and indicated no duplication of effort.

24. (U) A maximum of 20 patients with 20-30% burns will be entered into this study. The occurrence of grade 3 or 4 toxicity or a WBC count > 50,000/cubic mm of blood in at least 60% of the patients at any dose level will be used as evidence of toxicity. Treatment will continue for as long as the patient is hospitalized but will not exceed 2 months. Within a few days of stopping treatment, the patient will undergo a final examination. At this time, the patient will be evaluated with respect to complications of therapy, dose-limiting toxicity (if any), and adverse experiences and responses (if any).

25. (U) 8806 - 8809. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board and work will be initiated within the next few weeks.

DD FORM 1498, 83 MAR EDITION OF MAR 68 IS OBSOLETE

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: Phase II Study of Human Recombinant Granulocyte-Macrophage Colony-Stimulating Factor in Patients with Thermal Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
David G. Burleson, PhD, Lieutenant Colonel, MS
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Eric M. Bonnem, MD*

*Schering Corporation
Kenilworth, New Jersey 07033

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: Phase II Study of Human Recombinant Granulocyte-Macrophage Colony-Stimulating Factor in Patients with Thermal Injury

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
David G. Burleson, PhD, Lieutenant Colonel, MS
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Eric M. Bonnem, MD

The effect and safety of recombinant human granulocyte-macrophage colony-stimulating factor (rGM-CSF) is being assessed in patients with limited thermal injury. This study was designed to determine the safety of rGM-CSF in patients with thermal injury as well as investigate the effect of this drug on WBC subset population and function. Two patients out of a projected total of 20 patients have been enrolled in the study.

PHASE II STUDY OF HUMAN RECOMBINANT GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR IN PATIENTS WITH THERMAL INJURY

Thermal injury remains a significant clinical problem. Although early mortality from thermal injury has declined over the last several years, late mortality remains high, primarily due to infection. Despite improvements in nutrition, excision and skin grafting techniques, and aggressive fluid management, no significant impact has been made upon the defects in the immune system and the clinical consequences which contribute to mortality.

Although the cause of this immune dysfunction is unknown, there is speculation that the burn eschar may release toxins into the plasma and be responsible for the defect (1,2). Whatever the etiology may be, defects, both qualitative and quantitative, are noted in nearly all aspects of the immune system (3-16).

Monocyte dysfunction is manifested by decreased migration and PHA hyporesponsiveness (3,4). Altered lymphocyte function has several aspects. There is a decrease in the absolute number of T cells. There is a reversal of the normal T cell helper/suppressor ratio in some reports, a decrease in polyclonal B cell proliferation, and a failure to process antigen, such as tetanus toxoid (5-9). Leukocytes, although frequently normal in number, are severely dysfunctional. These defects are manifested by defective migration, phagocytosis, and degranulation (13,14). In addition, burn serum contains an inhibitor of complement conversion (C3) which leads to opsonization failure and further inhibition of neutrophil function. Taken together, dysfunction of neutrophils may be the major factor leading to the predisposition towards fatal sepsis (16). Recent work has demonstrated that many patients who do not survive burn injury are monocytopenic and lymphopenic in comparison with groups of survivors. Comparison of serum levels of hematopoietic colony-stimulating factors showed distinct differences between survivors and nonsurvivors; nonsurvivors demonstrated an inappropriate lag in the generation of colony-stimulating factors early in the course of burn and an inappropriately low level despite the presence of documented sepsis. This inappropriate response led to a relative failure of granulocytosis despite the presence of infection. The nonsurviving group had a mean burn of 59% vs. 38% in the surviving group (17). Later studies have demonstrated that postburn serum inhibits the in vitro production of colony-stimulating factor by mononuclear cells (18).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a lymphokine discovered at the Walter and Eliza Hall Institute (Royal Melbourne Hospital, Australia) over 20 yr ago.

GM-CSF was so named because of its ability to preferentially stimulate granulocytes and macrophages. Not only does GM-CSF have a proliferative potential for marrow progenitors, it can also stimulate various functional activities of monocytes and granulocytes. Macrophages are stimulated to secrete plasminogen activator and exhibit increased phagocytic and cytocidal activity for bacteria, yeast (19,20), and malignant cell lines (21). Granulocytes increase RNA and protein synthesis and exhibit increased antibody-dependent cytotoxic killing of tumor cells and enhanced oxidative metabolism (19,22-25). Many of these functions have been confirmed with human recombinant GM-CSF (rGM-CSF) (26). rGM-CSF stimulates mature neutrophils and eosinophils to augment cell surface antigenic expression and prolongs their survival (27). In addition, increases in phagocytic activity, synthesis of biologically active molecules, and augmented antibody-dependent cytotoxicity and expression of various cell surface markers are seen (19,25,28).

The gene for murine GM-CSF was cloned in 1984 (29). This, in turn, led to the isolation and expression of cDNA-mGM-CSF for human GM-CSF in 1985 (30-32). Early studies of rGM-CSF administered as a continuous infusion in primates resulted in a dramatic leukocytosis and reticulocytosis. Salutary effects have been observed in the pancytopenic rhesus and cynomolgus monkeys. Adverse effects were minimal at all doses (19,25,28,33-34). Administration of rGM-CSF to humans with an HIV infection resulted in minimal adverse reactions. As predicted, total WBC counts were elevated, with the largest increase in the absolute granulocyte count. The only adverse effects directly attributable to rGM-CSF were local phlebitis in 4 of 16 patients, transient skin rash, and facial flushing (35).

MATERIALS AND METHODS

Study Design. This is an open-label pilot study to determine the safety of rGM-CSF (SCH 39300, Schering Corporation, Kenilworth NJ) in patients with thermal injury > 20% and < 40% of the total body surface area. In addition, blood cells from a small number of patients will undergo *in vitro* laboratory testing of granulocyte, lymphocyte, and monocyte function. The major end point of the trial is to determine the safety of this drug in a population of patients with thermal injury. The study end point is the occurrence of a Grade 3 or 4 toxicity or a WBC count > 50,000/mm^3 of blood in at least 60% of patients at any dose level.

This study seeks to elicit any clinical or objective reactions from the patients being treated and to determine the relationship to rGM-CSF. Special attention is directed at detecting any adverse reactions. Potential clinical reactions can include headache, fatigue, neuropathy, confusion, altered level of consciousness, hypersensitivity reactions, anorexia,

nausea, vomiting, abnormal taste, flu-like symptoms, and bone pain. Potential objective reactions can include elevated liver enzymes, abnormal clotting times, cardiac arrhythmias, hypotension, and thromboembolic phenomenon. The reactions, if present, and any underlying disease are graded on the following scale: 1 = mild, 2 = moderate, 3 = severe, and 4 = life-threatening. In addition, the duration of any clinical or objective reaction is noted and whether intermittent or continuous.

Any patient who experiences Grades 3 or 4 toxicity which is attributable to the rGM-CSF and not the underlying disease receives no further rGM-CSF until resolution of the adverse reaction. Upon discussion with the sponsor, the patient can be retreated after resolution of the toxicity at 50% of the prior dose Recurrence of the same toxicity despite dose reduction requires withdrawal from the study. Patients who experience Grades 1 or 2 adverse reactions, with the exception of hepatic toxicity, may continue treatment at the same dose as long as there is no further progression to Grade 3 or 4 toxicity.

Drug. Recombinant DNA GM-CSF is a biologic material which is expected to have adverse reactions similar to other lymphokines, particularly at higher doses. Each patient enrolled in the study receives rGM-CSF (3, 10, or 15 mcg/kg IV) daily over a 4-h period. Treatment with rGM-CSF does not exceed the period of hospitalization or 2 months, whichever comes first.

Number of Patients. A maximum of 20 patients will be enrolled in the study.

Inclusion Criteria. The following patients are eligible for entry into the study:

1. Male or female patients > 18 yr of age. Female patients are either previously surgically sterilized or postmenopausal (> 45 yr of age and no menstrual periods for at least 1 yr) or have a negative pregnancy test result prior to initiation into the study.

2. Patients admitted to the Institute within 48 h of burn injury.

3. Patients with burn wounds > 20% and < 40% of the total body surface area, with at least 5% full-thickness injury.

Exclusion Criteria. The following patients are excluded from the study:

1. Patients < 18 yr of age.

2. Patients who are pregnant or nursing.

3. Patients with burns $< 20\%$ or $> 40\%$ of the total body surface area or $< 5\%$ full-thickness injury.

4. Patients with inhalation injury.

5. Patients receiving prophylactic antibiotics or corticosteroids.

6. Patients with a history of prior treatment with any lymphokines.

Patient Procedures. Once the patient has met all criteria of eligibility and informed consent has been obtained, a complete history and physical examination are performed. Clinical history includes date and extent of thermal injury. All concurrent drugs administered are recorded. The following laboratory procedures are performed at appropriate pre- and posttreatment evaluation times:

1. Hematologic tests - WBC count and differential, hematocrit, hemoglobin, platelet count, and reticulocyte count.

2. Blood chemistries - Clotting studies, including PTT/PT, glucose, urea nitrogen, uric acid, total and direct bilirubin, alkaline phosphatase, lactic dehydrogenase, alanine aminotransferase, aspartate aminotransferase, creatinine, inorganic phosphorus, electrolytes, albumin, total protein, cholesterol, triglycerides, calcium, and neutralization factors.

3. Urinalysis - Microscopic examination, 24-h urine for protein, and creatinine clearance.

4. Twelve-lead EKG.

5. WBC subset count and function.

6. Pharmacokinetics.

7. Bone marrow aspirate.

Patients continue to receive routine burn care as well as supportive care for any medical problems arising during the study. Within a few days of stopping treatment, the patient undergoes a final examination. At this time, the patient is evaluated with respect to complications of therapy, dose-limiting toxicity (if any), and adverse experiences and responses (if any).

If the patient is withdrawn from the study, notation is made in the case report as to the reason for withdrawal.

Regardless of the reason for withdrawal, the day the patient is removed from the study is considered the final treatment visit. All examinations as previously listed are obtained at that time.

Study Analysis. Three to four patients will be enrolled in each dose group. No statistical comparisons among those groups are planned. Data will be summarized and listed.

RESULTS

Two patients have been enrolled in the study to date.

DISCUSSION

Upon enrollment of a projected 20 patients, the results will be analyzed.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA315359	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY NONE	4. KIND OF SUMMARY A	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	DA	170
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Evaluation of in vitro Cultivated Keratinocytes as Epithelial Autografts for the Closure of Burn Wounds

12. SUBJECT AREAS
06 05 Medicine and Medical Research 06 13 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Basil A. Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.0	0
89	1.5	105

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR BUESCHER, T M
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-4440
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) CIOFFI, W G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) MASON, A D

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Healing; (U) Keratinocytes; (U) Cell Cultures; (U) Skin; (U) Skin Graft; (U) Frozen Skin; (U) Volunteers:

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Adults; (U) RAII

23. (U) To evaluate cultured keratinocytes as grafts for epithelial closure of burn wounds. To identify technical and immunological requirements to establish banks of frozen histocompatible keratinocytes for wound coverage in burned soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) The possible utility of cultured keratinocytes will be established initially with cultured autologous keratinocytes. Keratinocytes will be cultured from biopsies taken early after admission of patients with large burns and limited unburned donor sites for standard partial-thickness autografts. If such grafts are deemed clinically useful, efforts will expand into investigations of allogeneic skin cultures.

25. (U) 8710 - 8809. Two patients have been entered into the study during this reporting period. After the entry of 10 patients into the study, the safety and efficacy of the cultured keratinocytes will be assessed. This project was transferred from DA311491.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * USGPO. 1986 -491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG6972	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	F	301
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89-01			

11. TITLE (Precede with Security Classification Code) (U) Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury

12. SUBJECT AREAS
06 05 Medicine and Medical Research 06 13 Microbiology 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
76 10	99 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Basil A. Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.5	269
89	0.5	282

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, A T
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-3411
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Pseudomonas; (U) Klebsiella; (U) Staphylococcus; (U) Wound Infection; (U) Antibiotic Resistance;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Sepsis; (U) Topical Chemotherapy; (U) Volunteers: (U) Adults; (U) Children; (U) Lab Animals: (U) Rats; (U) Guinea Pigs; (U) Mice; (U) RAII

23. (U) Burns constitute a large component of military injuries sustained in combat. Military relevance of this research lies in the fact that infection and ensuing sepsis are major problems among burned soldiers. Control of surface infection is a major objective and species of organisms causing sepsis, epidemiology, response of significant species to topical chemotherapy modalities, and relationship of antibiotics to sepsis control are major study areas. A literature search was performed and indicated no duplication of effort.

24. (U) Cultures of human wounds, tissues, and body fluids are carried out with precise strain speciation and differentiation being employed. Virulence is assessed in burn wound models which are also used to assess effectiveness of experimental drugs, both topical and systemic.

25. (U) 8701 - 8712. During calander year 1987, microbiologic surveillance was carried out on 214 of the 221 admitted and discharged burn patients. More than 9,964 isolates were identified from 10,570 specimens. Gram-negative organisms represented < 35% of isolates. Klebsiella pneumoniae was the most common Gram-negative isolate. The most common blood isolate was Staphylococcus aureus. Pseudomonas aeruginosa was recovered from the blood of one patient. No Pseudomonas aeruginosa wound infections were identified.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * U.S.G.P.O. 1986-491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 January 1987 - 31 December 1987

INVESTIGATORS

Albert T. McManus, PhD
Jack R. Henderson, PhD
Timothy E. Lawson, Staff Sergeant
Charles H. Guymon, Sergeant
Aldo H. Reyes, Staff Sergeant
Pamela J. Luevano, Specialist
Bruce W. Tunell, Specialist
Michelle J. Tunell, Specialist
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Jan 87 through 31 Dec 87

INVESTIGATORS: Albert T. McManus, PhD
Jack R. Henderson, PhD
Timothy E. Lawson, Staff Sergeant
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Aldo H. Reyes, Staff Sergeant
Pamela J. Luevano, Specialist
Bruce W. Tunell, Specialist
Michelle J. Tunell, Specialist
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

During calendar year 1987, 214 burned patients were cultured and 9,966 isolates were identified. A relatively low colonization frequency (< 33%) with Gram-negative organisms has continued for the sixth reporting period. This was also reflected in an increase in Gram-positive organisms in blood cultures. Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus saprophyticus represented 39.6% of the bacteremia cases. The computerized microbial culture surveillance system now contains infection control and antibiotic usage data bases. This system is being evaluated for its use in predicting infecting organisms from previous sites of colonization and antibiotic usage.

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

This report is produced from microbiology data collected for patients admitted during calendar year 1987. Data were collected from admission through disposition. This is the third report that is based on calendar year rather than fiscal year. This change more nearly aligns culture results with the annual research progress report produced by the Clinical Division for the same patient population.

AUTOMATED MICROBIOLOGY DATA BASE

The microbiology data base now contains complete surveillance data for > 1,200 burn patient admissions. Epidemiologic use of these data has resulted in several publications. The microbiology data base has been aligned with antibiotic use and infection control data bases. This has improved the utility of the system for prospective use in identifying outbreaks and aiding empiric therapy by predicting on a statistical basis the probable antibiotic sensitivity patterns of infecting organisms.

ANTIBIOTIC SENSITIVITY DETERMINATION

The 1987 antibiotic testing panels are presented in Table 1. Bacterial organisms were tested by agar overlay disc diffusion. Broth dilution minimal inhibitory concentrations and minimal bactericidal concentrations were available upon specific request. The protocol for selecting organisms for *in vitro* sensitivities was isolation from blood cultures, predominant organisms in biopsy cultures, predominant Gram-negative organisms in sputum and urine cultures with $>10^5$ cfu/ml, *Staphylococcus aureus* isolates, *Pseudomonas aeruginosa* isolates, and other organisms as requested.

MICROBIAL SURVEILLANCE

The microbial surveillance protocol established during fiscal year 1983 was continued during calendar year 1987 (1). Patients were cultured from wound, sputum, urine, and rectum on admission. Thereafter, sputum and urine were cultured 3X/week and stools and wound surfaces 2X/week. Patients transferred to the convalescent ward and hospitalized > 30 days were cultured 1X/week. Gentamicin-resistant Gram-negative organisms from sputum or stool specimens were screened by plating on MacConkey agar containing gentamicin sulfate (25 μg/ml).

MICROBIOLOGIC FINDINGS IN BURN PATIENTS

A total of 214 patients admitted during 1987 were cultured. Species isolated and number of patients yielding each species are presented in Table 2. Because of the decreased host

TABLE 1. In vitro Sensitivity Panels (1987)

Enteric Organisms	Nonenteric Gram-Negative Organisms	Gram-Positive Organisms
1. Amikacin[a,b]	1. Amikacin[a,b]	1. Amikacin[a,b]
2. Gentamicin[a,b]	2. Gentamicin[a,b]	2. Gentamicin[a,b]
3. Ticarcillin[a]	3. Tobramycin	3. Tobramycin
4. Mezlocillin[a,b]	4. Ticarcillin[a]	4. Mezlocillin[b]
5. Piperacillin[a,b]	5. Mezlocillin[a,b]	5. Piperacillin[a,b]
6. Cefotaxime[a]	6. Piperacillin[a,b]	6. Moxalactam[b]
7. Cefoperazone	7. Moxalactam[a,b]	7. Cefotaxime
8. Sulfadiazine	8. Cefotaxime[a]	8. Cefoperazone
9. Netilmicin[a]	9. Cefoperazone[a]	9. Cefsulodin
10. Kanamycin	10. Cefsulodin[a]	10. Sulfadiazine
11. Chloramphenicol	11. Colistin	11. Oxacillin[a]
12. Tetracycline	12. Sulfadizine[a]	12. Cephalothin[a]
13. Cefoxitin[a]	13. Netilmicin	13. Vancomycin[a,b]
14. Cefamandole[a]	14. Kanamycin	14. Chloramphenicol[a]
15. Ampicillin[a]	15. Chloramphenicol	15. Tetracycline[a]
16. Trimethoprim	16. Tetracycline	16. Ampicillin
17. Trimeth and Sulfa	17. Imipenem-Cilastatin[b]	17. Imipenem-Cilastatin[b]
18. Nalidixic Acid	18. Azlocillin[a]	18. Clindamycin[a]
19. Imipenem-Cilastatin[b]	19. Norfloxacin	19. Penicillin[a]
20. Streptomycin	20. Aztreonam	20. Erythromycin[a]
21. Aztreonam	21. Timentin	21. Streptomycin
22. Norfloxacin	22. Ceftazidime[a,b]	22. Ceftazidime[a,b]
23. Ceftazidime[a,b]	23. Ceftriaxone	23. Ceftriaxone
24. Ceftriaxone[a]		

[a]Reported daily on daily clinical microbiology report (hard copy).
[b]Reported on computer screen from patient data base.

TABLE 2. Distribution by Organism (1987)

Organism	Number of Isolates	Number of Patients Colonized	Organism	Number of Isolates	Number of Patients Colonized
Acinetobacter anitratus	25	7	Morganella morganii	59	19
Acinetobacter lwoffii	5	5	Neisseria lactamicas	4	4
Aeromonas hydrophila	10	3	Neisseria mucosa	462	122
Alcaligenes faecalis	7	4	Neisseria subflava	1	1
Alcaligenes xylosoxydans	2	2	Propionibacterium acnes	2	2
Arizona species	1	1	Proteus mirabilis	26	46
Aspergillus flavus	15	6	Proteus vulgaris	2	2
Aspergillus niger	3	2	Proteus rettgeri	5	4
Bacillus	31	25	Providencia alcalifac	1	1
Bacteroides species	1	1	Pseudomonas aeruginosa	403	55
Bordetella bronchiseptica	1	1	Pseudomonas *cepacia*	1	1
Branhamella catarrhalis	12	8	Pseudomonas maltophila	12	4
Candida albicans	220	42	Pseudomonas putida	7	7
Candida rugosa	42	6	Serratia liquefaciens	1	1
Candida tropicalis	15	9	Serratia marcescens	26	10
Citrobacter freundii	24	10	Staphylococcus aureus	1,786	150
Citrobacter diversus	56	10	Staphylococcus epidermidis	504	146
Citrobacter species	3	3	*Staphylococcus saprophyticus*	165	67
Clostridium difficile	1	1	Alpha Streptococcus	18	15
Clostridium haemolyticum	1	1	Beta hemolytic Streptococcus	4	3
Corynebacterium xerosis	4	2	Beta Streptococcus, Not	114	43
Corynebacterium species	3	3	Group A, B, or D		
Enterobacter aerogenes	265	51	Group A Streptococcus	1	1
Enterobacter agglomerans	18	10	Group A nonhemolytic beta	1	1
Enterobacter cloacae	203	47	Streptococcus		
Enterobacter species	3	3	Group B Streptococcus	20	5
Escherichia coli	444	91	Group D Streptococcus, not	253	87
Gram-positive cocci	2	1	Enterococcus		
Gram-positive rod	2	2	Group D Enterococcus	422	74
Gram-positive rod, anaerobic	1	1	Group F Streptococcus	2	1
Haemophilus influenzae	18	9	Nonhemolytic Streptococcus	16	15
Haemophilus species	1	1	Nonhemolytic Streptococcus,	1,231	197
Hafnia alvei	4	1	not Group D		
Klebsiella oxytoca	54	23	Streptococcus pneumoniae	24	15
Klebsiella ozaenae	2	1	Streptococcus viridans	1,929	208
Klebsiella pneumoniae	650	99	True fungi species (Other)	51	25
Micrococcus luteus	2	2	Yeast species (Other)	42	19

Total Number of Isolates = 9,966

Total Number of Cultured Patients = 214

resistance of the patient population, no organism is considered "normal" flora and all isolated organisms are reported to the physician. A summary of the 10 most common isolates is presented in Table 3. The table contains 79% of the species identified. The relative frequencies of sites of isolation are presented in Figure 1. The relative frequencies of sites of isolation of Gram-negative organisms, Gram-positive organisms, and yeast are shown in Figure 2.

FLORA RECOVERED FROM RESPIRATORY SYSTEM SPECIMENS

A total of 7,091 organisms were recovered from respiratory system specimens. The majority of these were sputum cultures collected in the surveillance program. The 10 most frequent species are presented in Table 4, which represents 85.9% of the respiratory isolates. Of particular note is the continued decline of Gram-negative isolates. Pseudomonas aeruginosa was not in the top 10 organisms, with only 25 of the 207 patients colonized. This frequency was not significantly different from calendar years 1984-86.

FLORA RECOVERED FROM WOUND SURFACE SPECIMENS

A total of 1 441 contact plate surface cultures were taken and 606 isolates were made. Relative frequencies of isolated species are presented in Figure 3. Subsurface flora, as measured by biopsy specimens, was measured in 214 biopsies taken from 40 patients. Organisms were recovered from 37 of the biopsied patients. The 10 most common organisms are presented in Table 5. Filamentous fungi remained the principal isolate with Aspergillus being the most common fungal genus. Pseudomonas aeruginosa was recovered from 9 biopsies taken from 5 patients. The continued decrease in recovery of wound bacteria is best correlated with the decrease in resistance to topical and parenteral antimicrobial agents. The loss of competitive bacterial flora is a reasonable basis for the increased frequency of fungal isolates.

FLORA RECOVERED FROM URINARY TRACT SPECIMENS

Urine specimens from 213 patients yielded 969 isolates. The 10 most common species are presented in Table 6. The top 10 organisms isolated from urine specimens with $>10^5$ cfu/ml are presented in Table 7.

FLORA RECOVERED FROM BLOOD CULTURES

Blood cultures were obtained from 115 patients for a total of 773 cultures. The principal organisms recovered are listed in Table 8. Positive cultures were obtained from 32 patients and 103 isolates were made from 100 positive cultures. Fifty-three cases of bacteremia were noted. A case of bacteremia was defined as isolation of an organism once or more than once with a 30-day period.

TABLE 3. Ten Most Frequent Isolates (1987)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	208	97.2	1,929	19.4
Nonhemolytic Streptococcus, not Group D	197	92.1	1,231	12.4
Staphylococcus aureus	150	70.1	1,786	17.9
Staphylococcus epidermidis	146	68.2	504	5.1
Neisseria mucosa	122	57.0	462	4.6
Klebsiella pneumoniae	99	46.3	650	6.5
Escherichia coli	91	42.5	444	4.5
Group D Streptococcus, not Enterococcus	87	40.7	253	2.5
Group D Enterococcus	74	34.6	422	4.2
Staphylococcus saprophyticus	67	31.3	165	1.7

Total Number of Patients Cultured = 214
Total Number of Isolates = 9,966

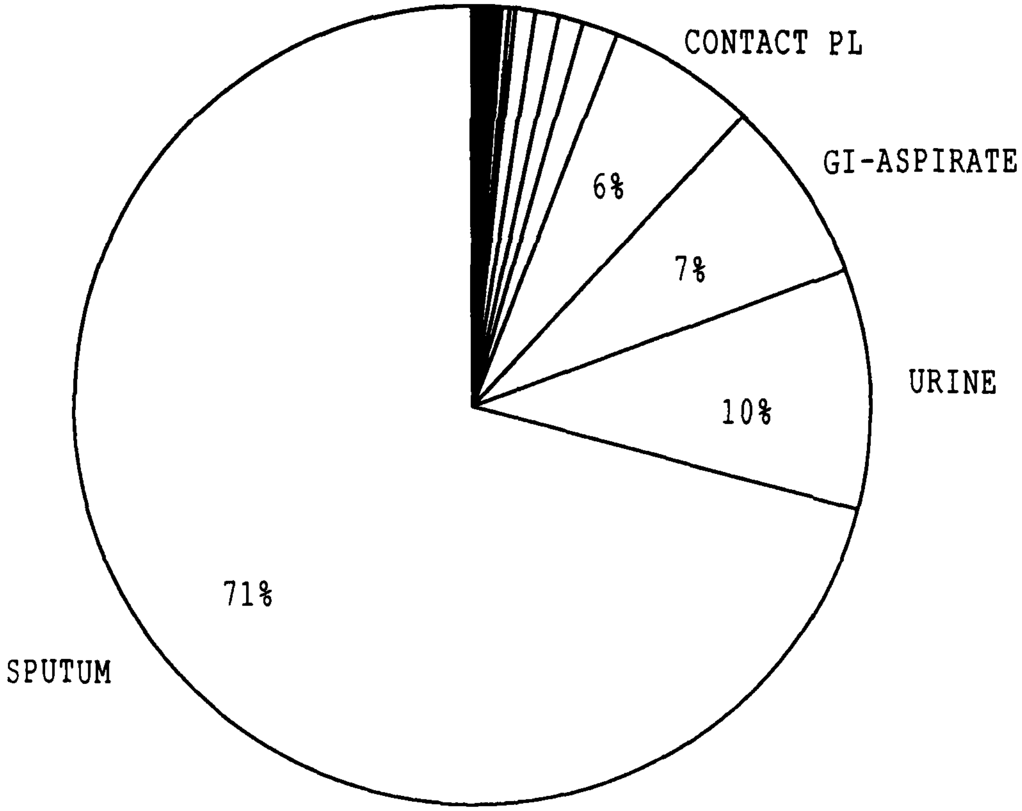


FIGURE 1. Display of the relative frequency of specimen sources yielding isolates in 1987.

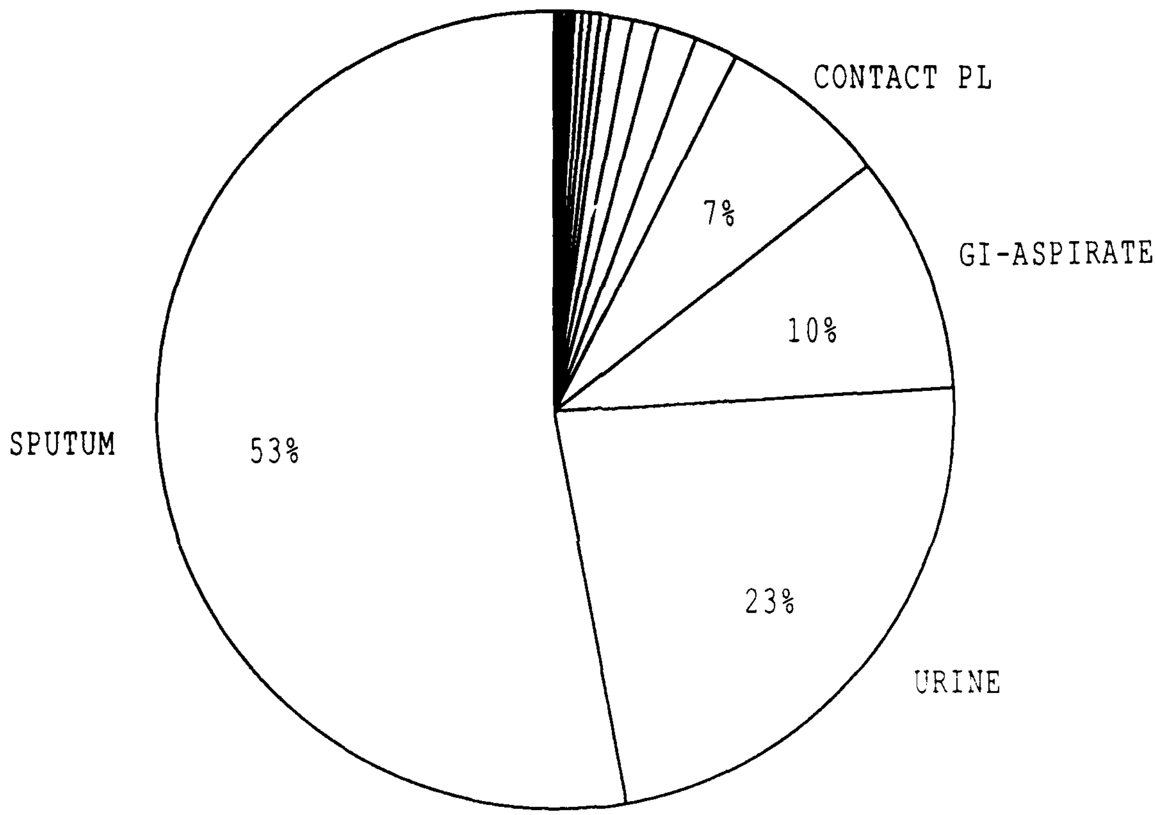


FIGURE 2A. Display of the relative frequency of specimen sources yielding Gram-negative organisms.

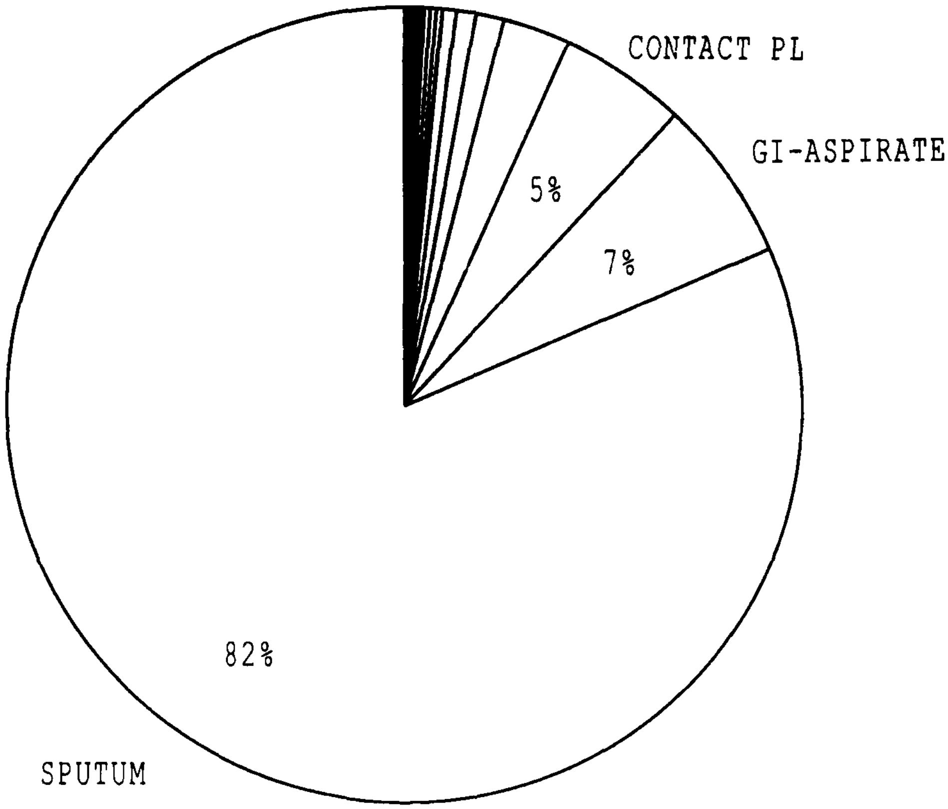


FIGURE 2B. Display of the relative frequency of specimen sources yielding Gram-positive organisms.

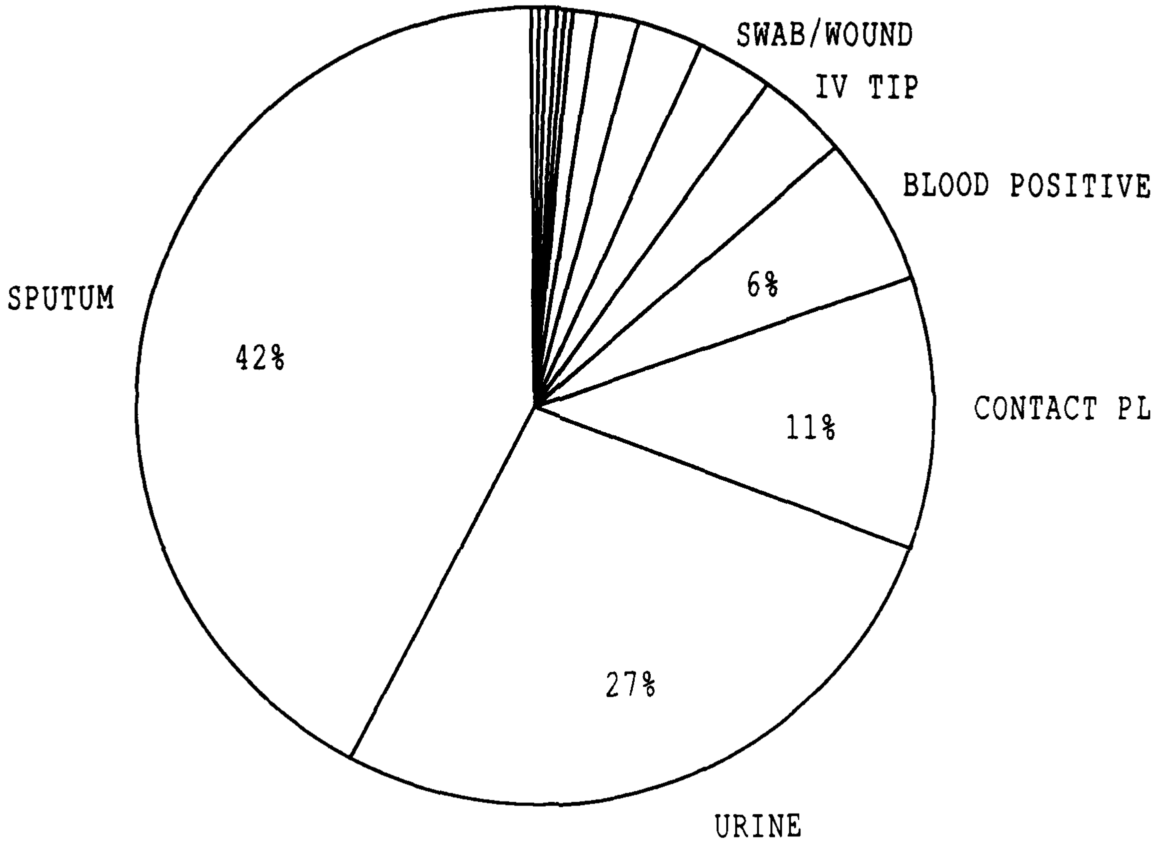


FIGURE 2C. Display of the relative frequency of specimen sources yielding yeast-like organisms.

TABLE 4. Ten Most Frequent Isolates from Respiratory Sources (1987)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	207	100.0	1,819	25.7
Nonhemolytic Streptococcus, not Group D	192	92.8	1,101	15.5
Staphylococcus aureus	132	63.8	1,334	18.8
Neisseria mucosa	121	58.5	435	6.1
Staphylococcus epidermidis	104	50.0	333	4.7
Group D Streptococcus, not Enterococcus	86	41.5	402	5.7
Staphylococcus saprophyticus	50	24.2	117	1.6
Klebsiella pneumoniae	44	21.3	288	4.1
Beta hemolytic Streptococcus, not Group A, B, or D	41	19.8	100	1.4
Escherichia coli	39	18.8	163	2.3

Total Number of Patients Cultured = 207
Total Number of Isolates = 7,091

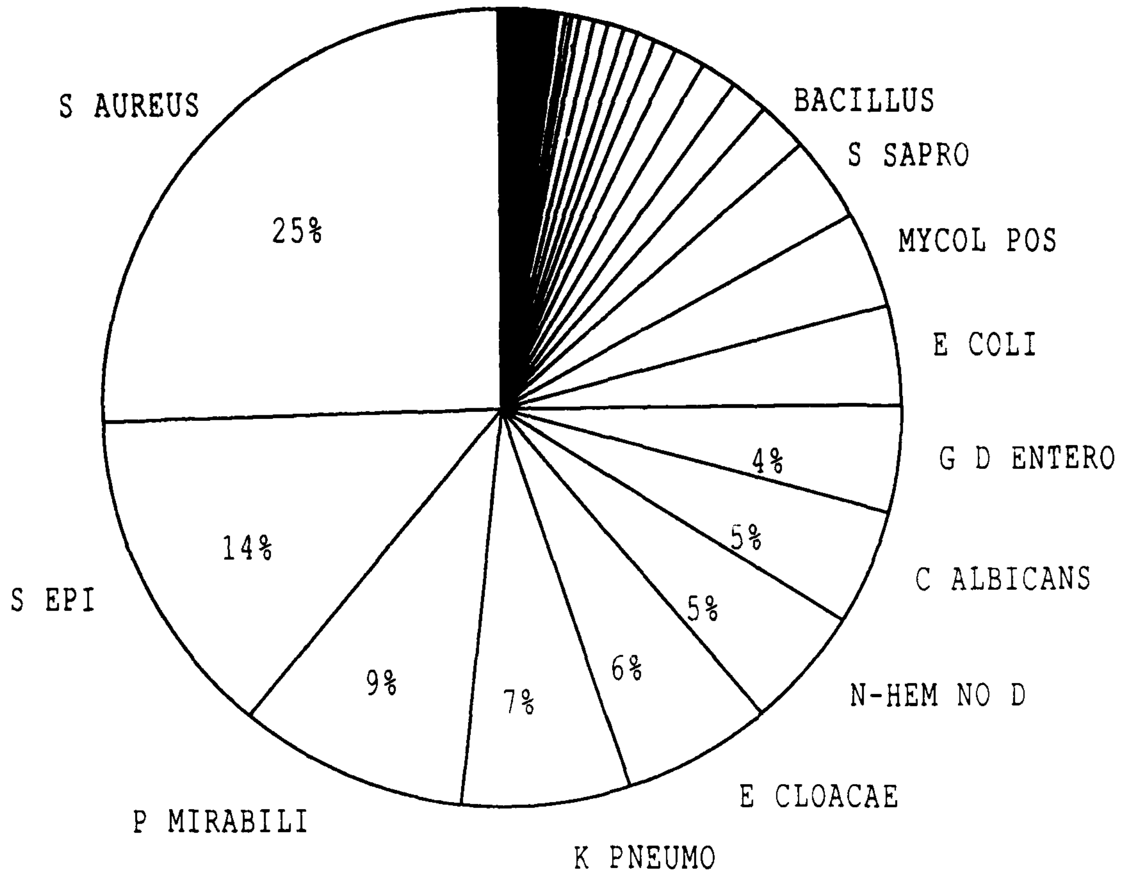


FIGURE 3. Display of the relative frequency of organism types isolated from surface wound cultures.

TABLE 5. Principal Organisms Recovered in Biopsy Specimens (1987)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Filamentous fungi	14	35.0	39	31.5
Staphylococcus aureus	8	20.0	13	10.5
Escherichia coli	7	17.5	13	10.5
Nonhemolytic Streptococcus, not Group D	5	12.5	5	4.0
Pseudomonas aeruginosa	5	12.5	5	4.0
Candida albicans	4	10.0	5	4.0
Enterobacter cloacae	3	7.5	5	4.0
Group D Enterococcus	3	7.5	10	8.1
Klebsiella pneumoniae	3	7.5	7	5.6
Candida tropicalis	2	5.0	2	1.6

Total Number of Patients Biopsied = 40
Total Number of Isolates = 124
Biopsies Taken = 214

TABLE 6. Ten Most Frequent Organisms from Urinary Specimens (1987)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Klebsiella pneumoniae	64	30.0	188	19.4
Escherichia coli	58	27.2	192	19.8
Pseudomonas aeruginosa	30	14.1	84	8.7
Proteus mirabilis	29	13.6	122	12.6
Staphylococcus aureus	27	12.7	48	5.0
Nonhemolytic Streptococcus, not Group D	26	12.3	34	3.5
Group D Enterococcus	24	11.3	47	4.9
Enterobacter aerogenes	21	9.9	31	3.2
Candida albicans	19	8.9	46	4.7
Staplylococcus epidermis	15	7.0	18	1.9

Total Number of Patients Cultured = 213
Total Number of Isolates = 969

TABLE 7. Ten Most Frequent Organisms from Urinary Specimens with $>10^5$ cfu (1987)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Klebsiella pneumoniae	45	45.5	105	20.3
Escherichia coli	39	39.4	95	18.4
Pseudomonas aeruginosa	27	26.3	61	11.8
Proteus mirabilis	23	23.2	70	13.5
Nonhemolytic Streptococcus, not Group D	14	14.1	19	3.7
Enterobacter aerogenes	13	13.1	15	2.9
Group D Enterococcus	13	13.1	27	5.2
Staphylococcus aureus	13	13.1	18	3.5
Candida albicans	10	10.1	20	3.9
Morganella morganii	9	9.1	9	1.7

Total Number of Patients Cultured = 99
Total Number of Isolates = 517

TABLE 8. Principal Organisms Found in Blood Cultures (1987)

Organism	Number of Patients	% Patients Cultured	% Cases	Number of Isolates	% Total Isolates
Staphylococcus aureus	13	11.3	24.5	27	26.2
Staphylococcus epidermidis	5	4.3	9.4	7	6.8
Klebsiella pneumoniae	4	3.5	7.5	6	5.8
Candida rugosa	3	2.6	5.7	7	6.8
Klebsiella oxytoca	3	2.6	5.7	7	6.8
Staphylococcus saprophyticus	3	2.6	5.7	4	3.9
Candida albicans	2	1.7	3.8	11	10.7
Corynebacterium species	2	1.7	3.8	2	1.9
Enterobacter cloacae	2	1.7	3.8	5	4.9
Escherichia coli	2	1.7	3.8	2	1.9
Group D Enterococcus	2	1.7	3.8	4	3.9

Total Number of Patients Cultured = 115   Total Number of Cultures = 773
Total Number of Isolates = 103   Total Number of Patient Positives = 32

Intravenous catheter tips were cultured from 80 patients. Isolations were made from 47 patients and 135 isolates were made. Data are presented in Table 9. These data show an unexpectedly high incidence of contamination.

SUMMARY OF ANTIBIOTIC TESTING

A total of 3,786 bacterial isolates were tested for *in vitro* sensitivity to antibiotics. A comparison of sources of tested strains is presented in Figure 4. The relative frequency of tested organisms is presented in Figure 5.

Gentamicin resistance was again used as a plasmid surveillance marker. Testing was done on 3,586 isolates. Figure 6 displays the relative frequency of tested organisms. Figure 7 displays the frequency of resistant species. *Staphylococcus aureus* represented 94% of the gentamicin-resistant isolates. Only 100 Gram-negative isolates of 965 strains tested were resistant to gentamicin (10.4%). This is the lowest percentage ever reported from the Institute and is a direct marker of the success of infection control isolation techniques in preventing the accumulation of a resistant Gram-negative flora.

Staphylococcus aureus. The sources of *Staphylococcus aureus* strains tested for *in vitro* activity are presented in Figure 8. The incidence of multiply resistant *Staphylococcus aureus* was 46% of isolates and these strains were isolated from 83 patients. The resistant strains are multiply resistant, with expression of gentamicin, erythromycin, oxacillin, and streptomycin resistance. Multiply resistant *Staphylococcus aureus* and gentamicin-sensitive strains are displayed separately in Table 10 and histograms are shown in Figure 9.

Pseudomonas aeruginosa. The frequency of sources of *Pseudomonas aeruginosa* strains tested *in vitro* is presented in Figure 10. The results of testing are presented in Table 11. Sensitivity to aminoglycoside antibiotics has remained high. The relative frequency of gentamicin resistance for recent reporting periods is presented in Figure 11. The relative frequency of sulfonamide resistance for recent reporting periods is presented in Figure 12. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 13.

Klebsiella pneumoniae. A total of 409 isolates were tested for *in vitro* sensitivities to antibiotics. The sources of isolation for tested strains are presented in Figure 14. The results of *in vitro* antibiotic testing are presented in Table 12. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 15.

TABLE 9. Ten Most Frequent Organisms from Intravenous Catheters (1987)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Staphylococcus aureus	24	30.0	32	23.7
Staphylococcus epidermidis	20	25.0	22	16.3
Klebsiella pneumoniae	9	11.3	14	10.4
Group D Enterococcus	9	11.3	9	6.7
Enterobacter cloacae	5	6.3	8	5.9
Escherichia coli	5	6.3	6	4.4
Proteus mirabilis	5	6.3	8	5.9
Pseudomonas aeruginosa	5	6.3	5	3.7
Staphylococcus saprophylytic	4	5.0	5	3.7
Candida rugosa	3	3.8	4	23.0

Total Number of Patients Cultured = 80
Total Number of Isolates = 135

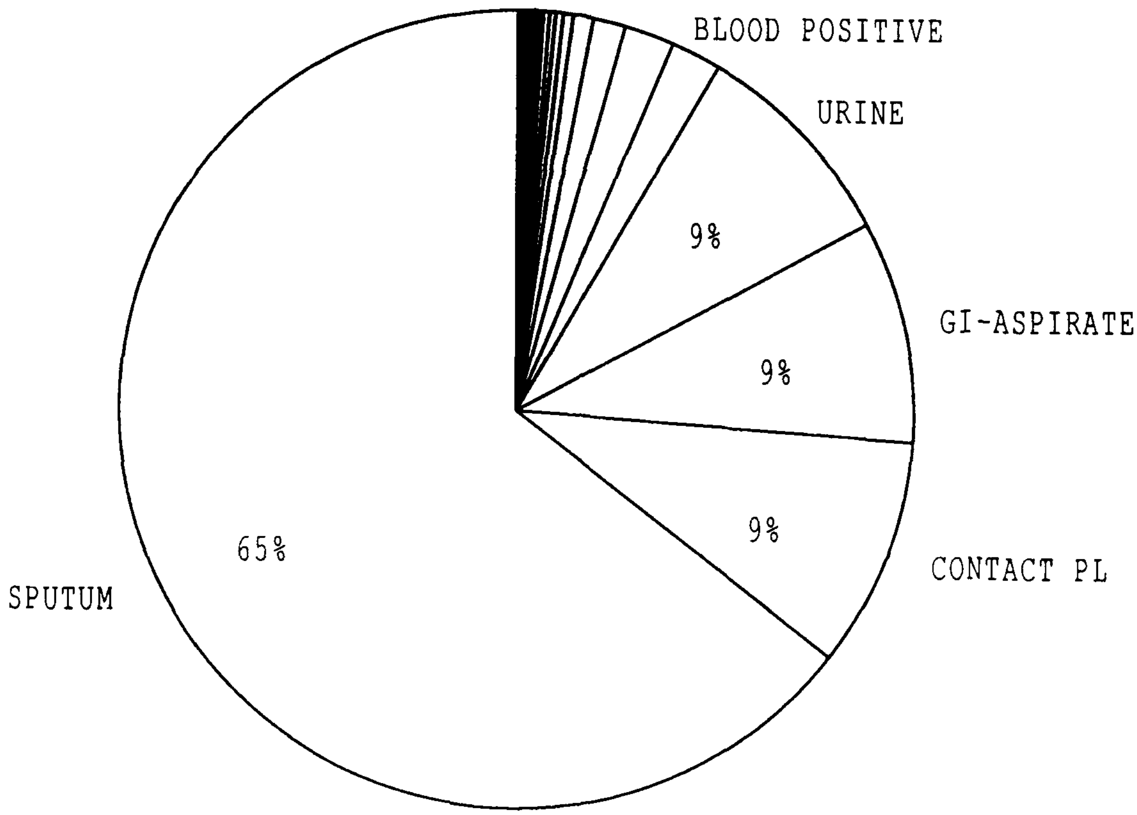


FIGURE 4. Display of the relativ^ frequency of sources yielding organisms tested .or *in vitro* sensitivity to antibiotics in 1987.

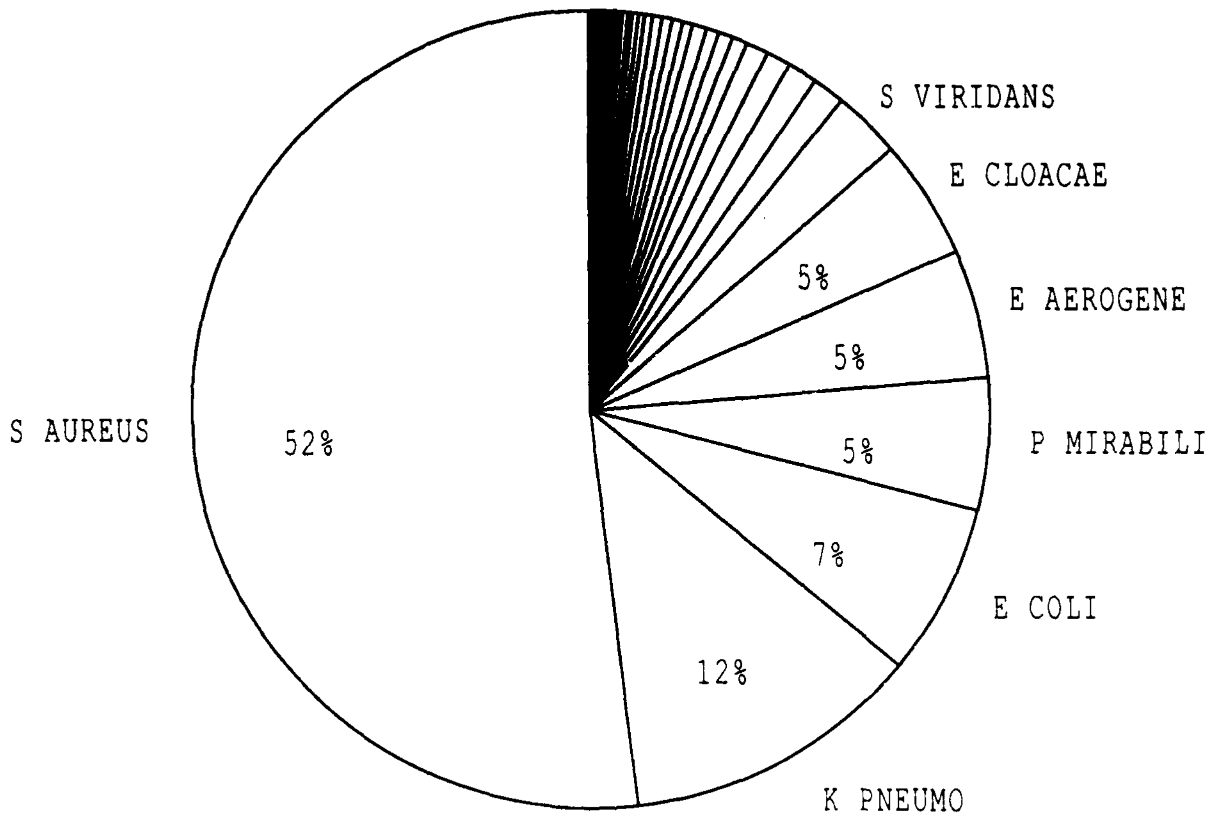


FIGURE 5. Display of the relative frequency of organisms tested for *in vitro* sensitivity to antibiotics in 1987.

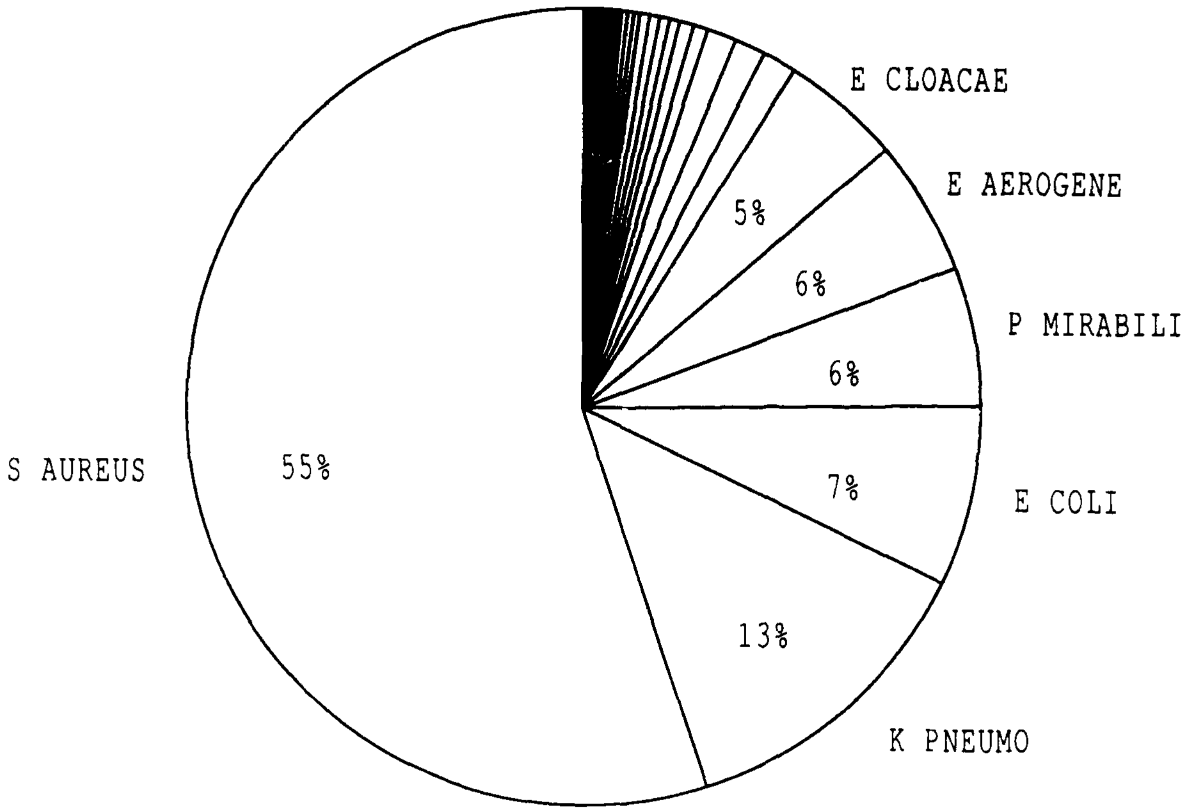


FIGURE 6. Display of the relative frequency of organisms tested for in vitro sensitivity to gentamicin in 1987.

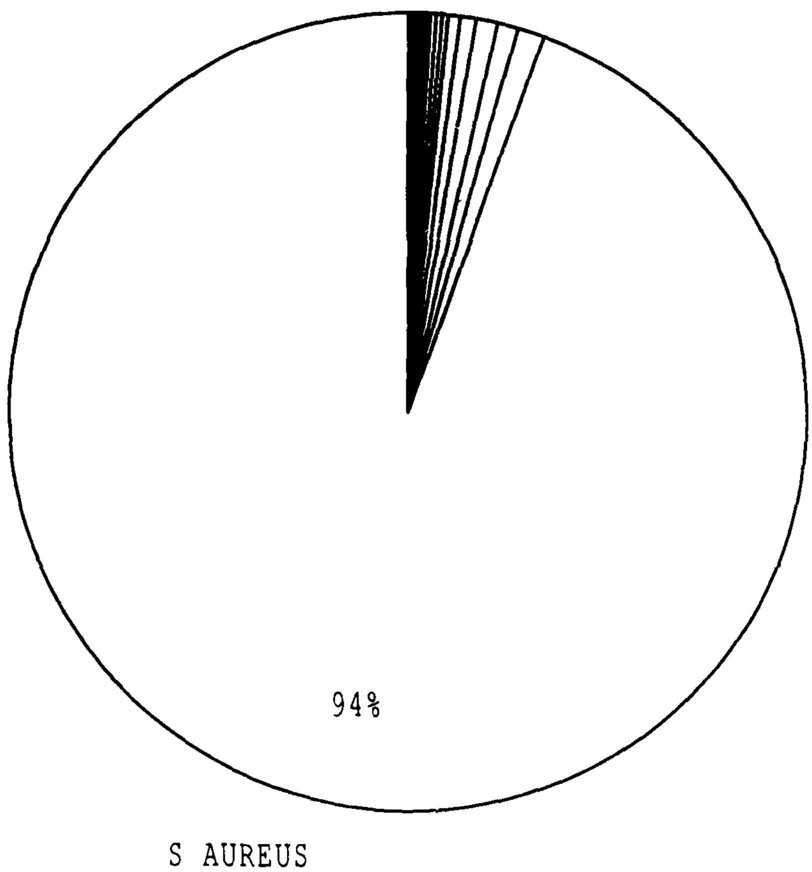


FIGURE 7. Display of the relative frequency of gentamicin-resistant organisms isolated in 1987.

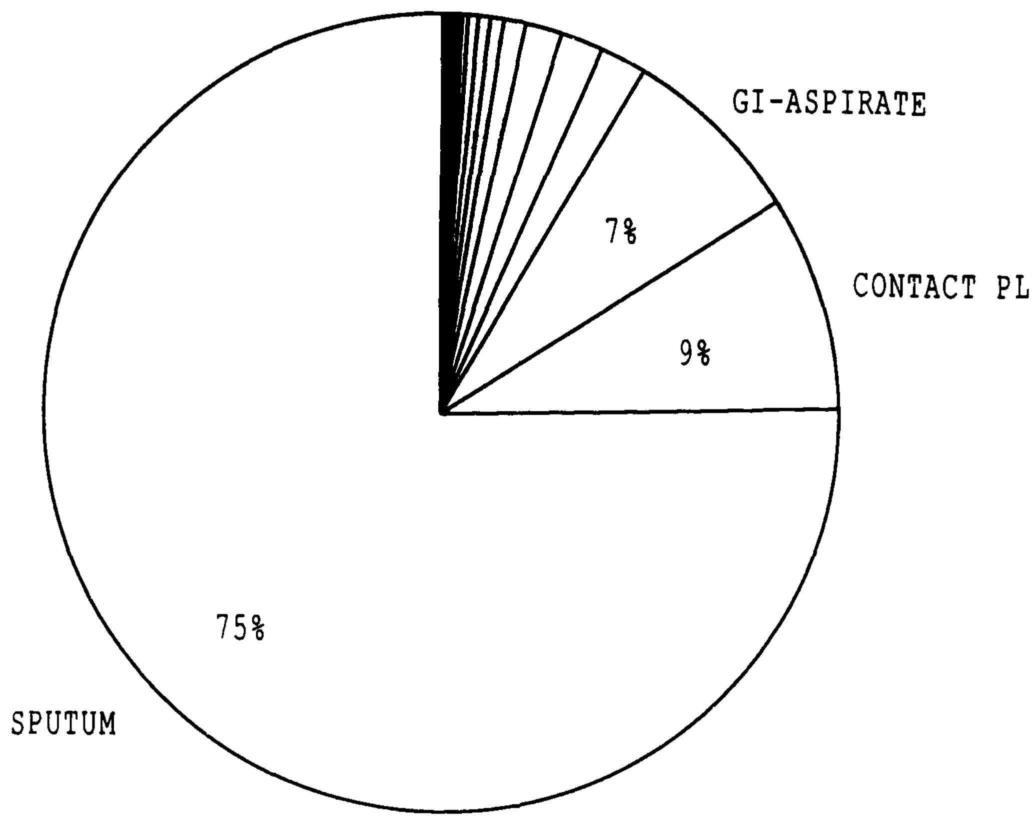


FIGURE 8. Display of the relative frequency of sources yielding <u>Staphylococcus aureus</u> tested for <u>in vitro</u> sensitivity to antibiotics in 1987.

TABLE 10A. Antibiotic Sensitivity Data for *Staphylococcus aureus* Sensitive to Gentamicin (1987)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	0.00	0	0.00	0	100.00	814	814
Ampicillin	1.90	18	8.35	79	89.75	849	946
Cefoperazone	0.25	2	14.15	115	85.61	696	813
Cefotaxime	1.58	15	0.84	8	97.57	924	947
Cefsulodin	0.93	5	0.37	2	98.70	532	539
Ceftazidime	0.00	0	0.00	0	100.00	765	765
Ceftriaxone	0.00	0	0.00	0	100.00	764	764
Cephalothin	0.11	1	0.21	2	99.68	945	948
Chloramphenicol	0.00	0	0.42	4	99.58	943	947
Clindamycin	2.23	21	0.21	2	97.56	920	943
Erythromycin	7.07	67	0.00	0	92.93	880	947
Gentamicin	0.00	0	0.32	3	99.68	945	948
Imipenem-cilastatin sodium	0.00	0	0.00	0	100.00	814	814
Kanamycin	0.00	0	19.05	8	80.95	34	42
Mezlocillin	2.85	27	32.91	312	64.24	609	948
Moxalactam	2.00	19	10.23	97	87.76	832	948
Oxacillin	2.43	23	2.75	26	94.83	898	947
Penicillin	85.23	808	7.07	67	7.70	73	948
Piperacillin	2.43	23	32.73	310	64.84	614	947
Streptomycin	0.99	8	0.37	3	98.64	796	807
Sulfadiazine	1.61	15	5.25	49	93.15	870	934
Tetracycline	8.48	80	0.95	9	90.56	854	943
Ticarcillin	0.00	0	0.00	0	100.00	43	43
Tobramycin	1.90	18	0.00	0	98.10	930	948
Vancomycin	0.00	0	0.00	0	100.00	945	945

TABLE 10B. Antibiotic Sensitivity Data for *Staphylococcus aureus* Resistant to Gentamicin (1987)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	1.75	13	11.46	85	86.79	644	742
Ampicillin	87.45	718	7.43	61	5.12	42	821
Cefoperazone	6.61	49	85.70	635	7.69	57	741
Cefotaxime	26.10	214	67.68	555	6.22	51	820
Cefsulodin	0.52	2	7.07	27	92.41	353	382
Ceftazidime	0.00	0	0.00	0	100.00	700	700
Ceftriaxone	0.00	0	0.00	0	100.00	705	705
Cephalothin	0.37	3	0.73	6	98.90	812	821
Chloramphenicol	3.41	28	1.71	14	94.88	779	821
Clindamycin	5.26	43	0.24	2	94.49	772	817
Erythromycin	12.55	103	1.58	13	85.87	705	821
Gentamicin	95.98	788	4.02	33	0.00	0	821
Imipenem-cilastatin sodium	0.13	1	0.27	2	99.60	739	742
Kanamycin	45.45	15	48.48	16	6.06	2	33
Mezlocillin	92.68	760	3.41	28	3.90	32	820
Moxalactam	91.34	749	4.63	38	4.02	33	820
Oxacillin	93.54	763	1.34	11	5.12	42	816
Penicillin	99.15	814	0.61	5	0.24	2	821
Piperacillin	93.18	765	3.05	25	3.78	31	821
Streptomycin	94.72	700	0.81	6	4.47	33	739
Sulfadiazine	71.75	569	20.43	162	7.82	62	793
Tetracycline	6.50	53	0.49	4	93.01	758	815
Ticarcillin	23.53	8	58.82	20	17.65	6	34
Tobramycin	96.47	792	2.07	17	1.46	12	821
Vancomycin	0.00	0	0.00	0	100.00	821	821

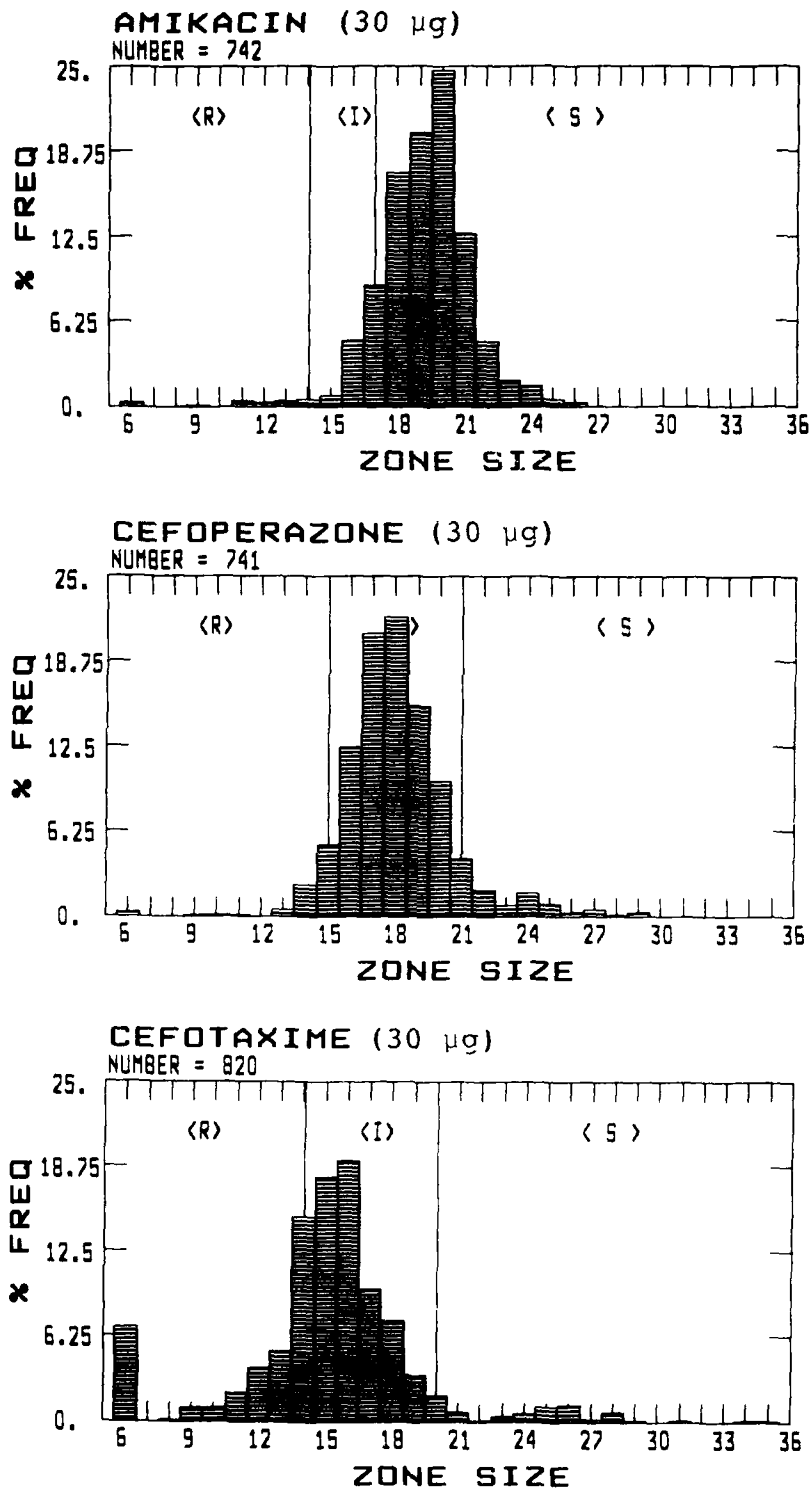


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant Staphylococcus aureus.

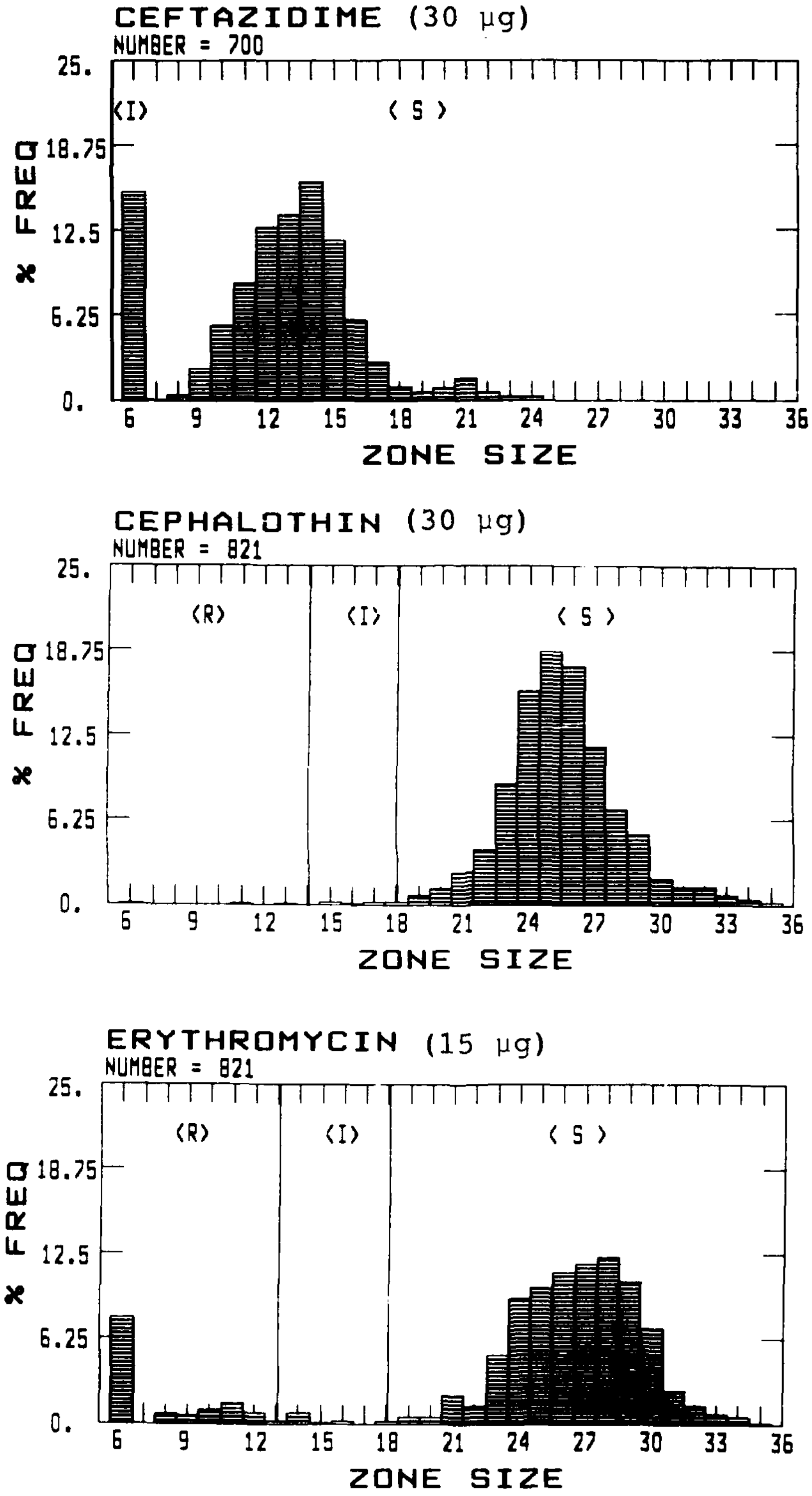


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).

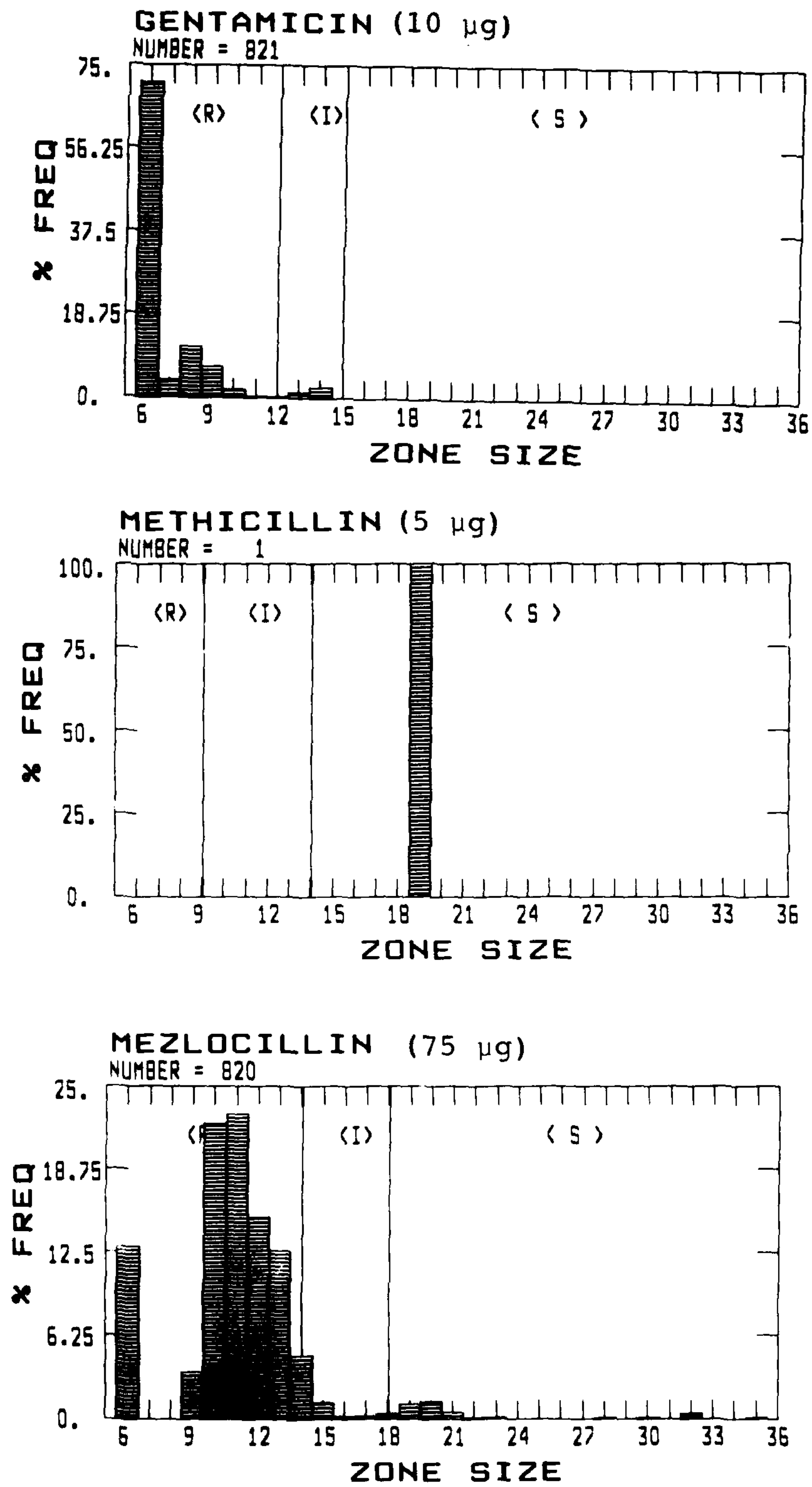
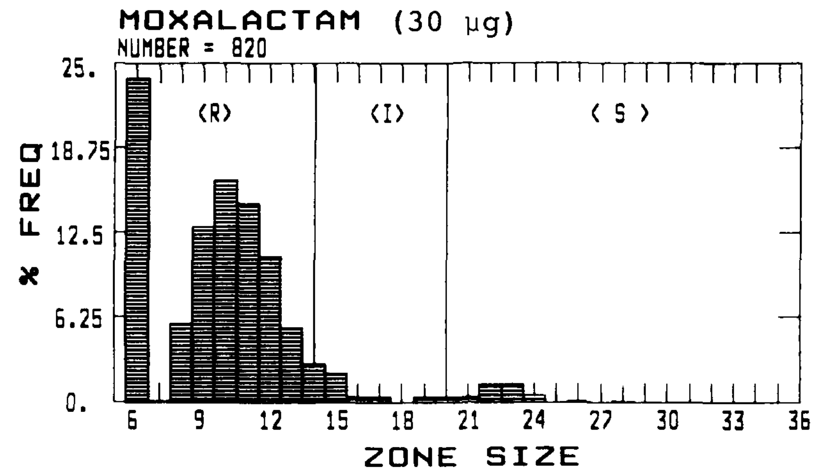
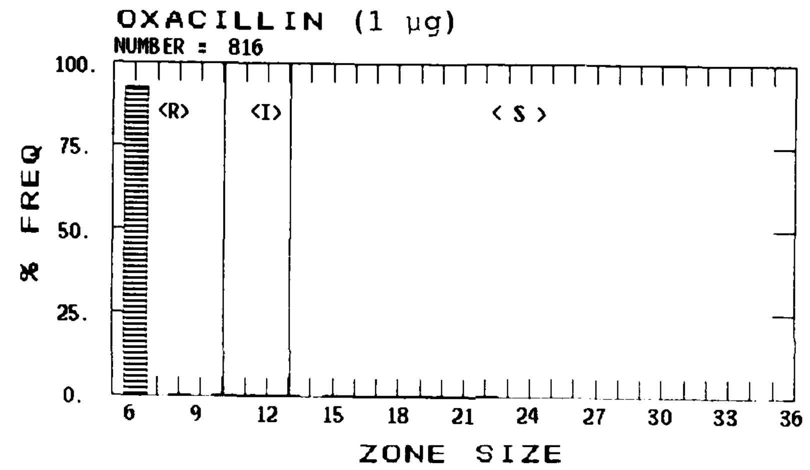


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant Staphylococcus aureus (continued).





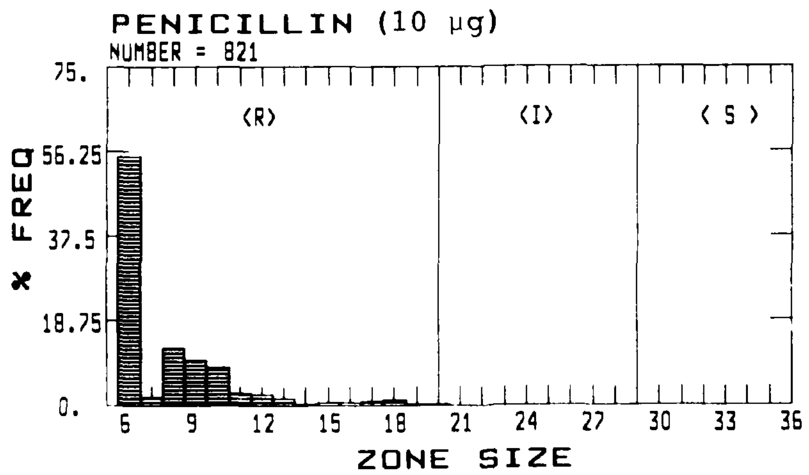


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant Staphylococcus aureus (continued).

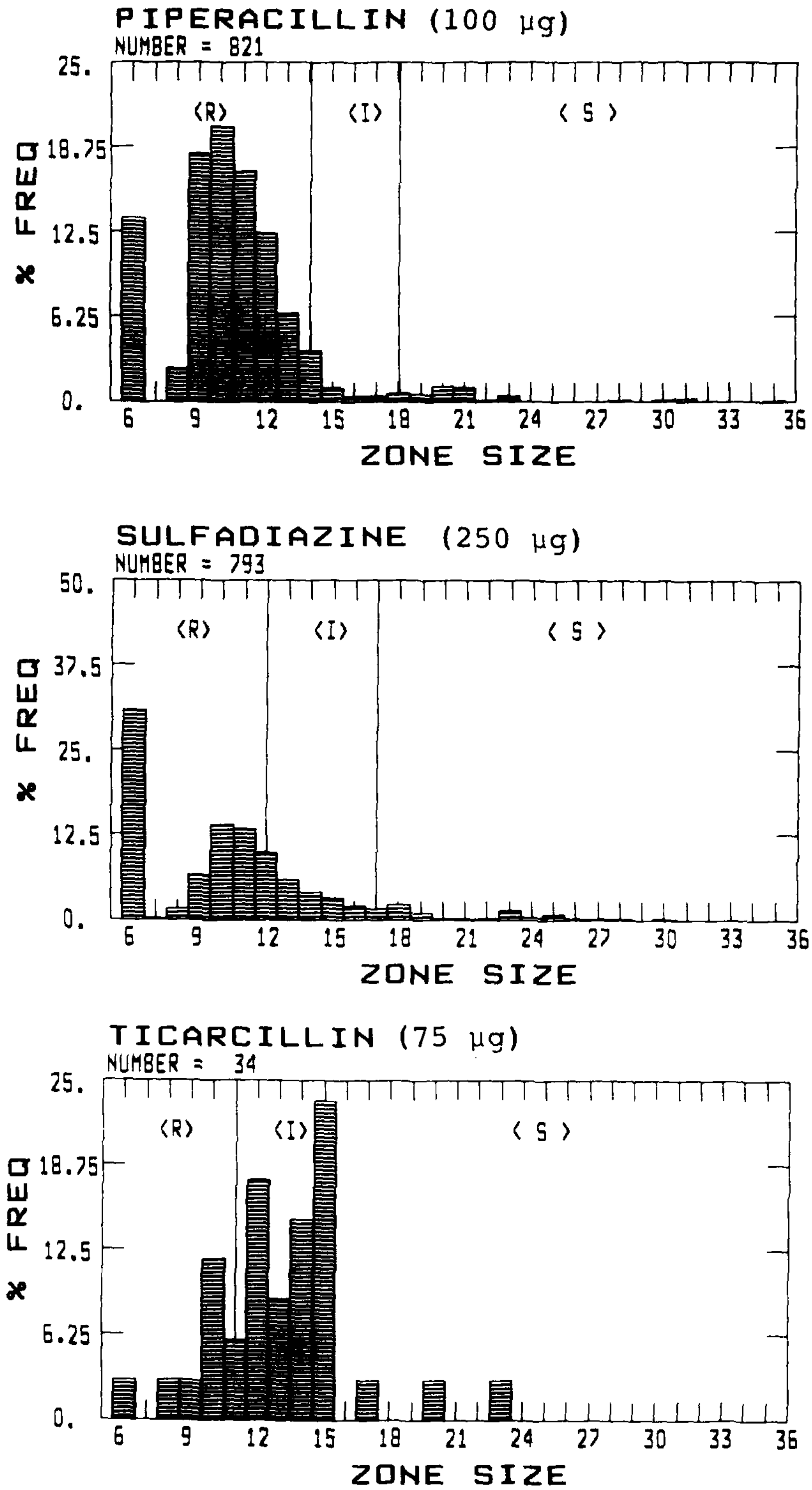
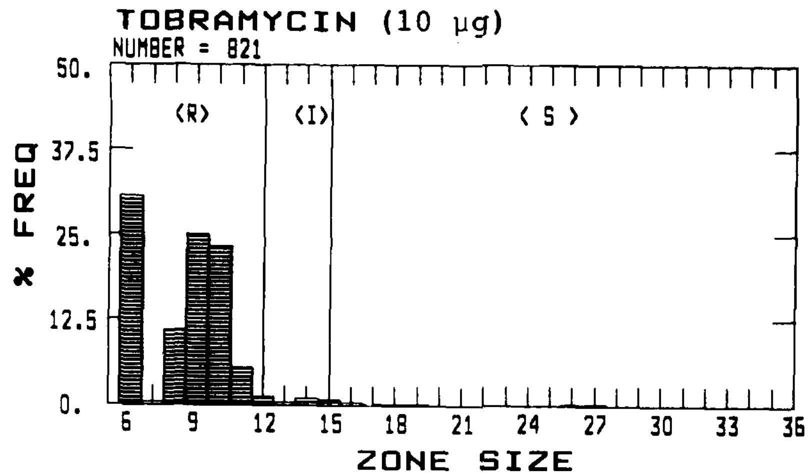


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant Staphylococcus aureus (continued).



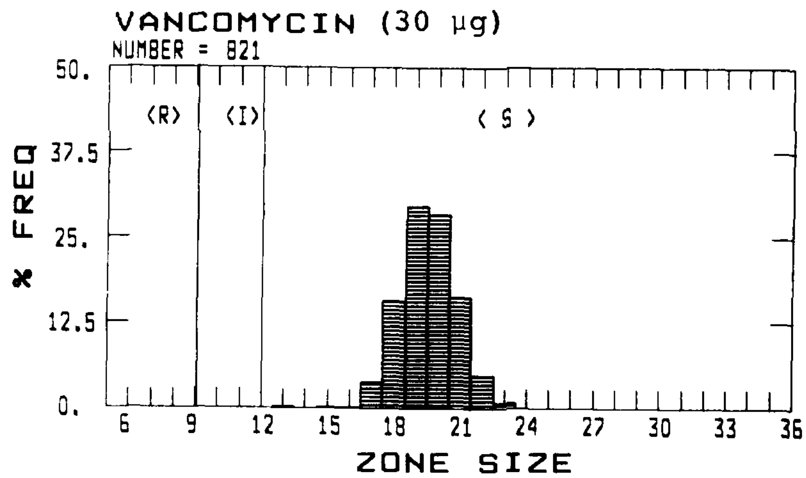


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant <u>Staphylococcus</u> <u>aureus</u> (continued).

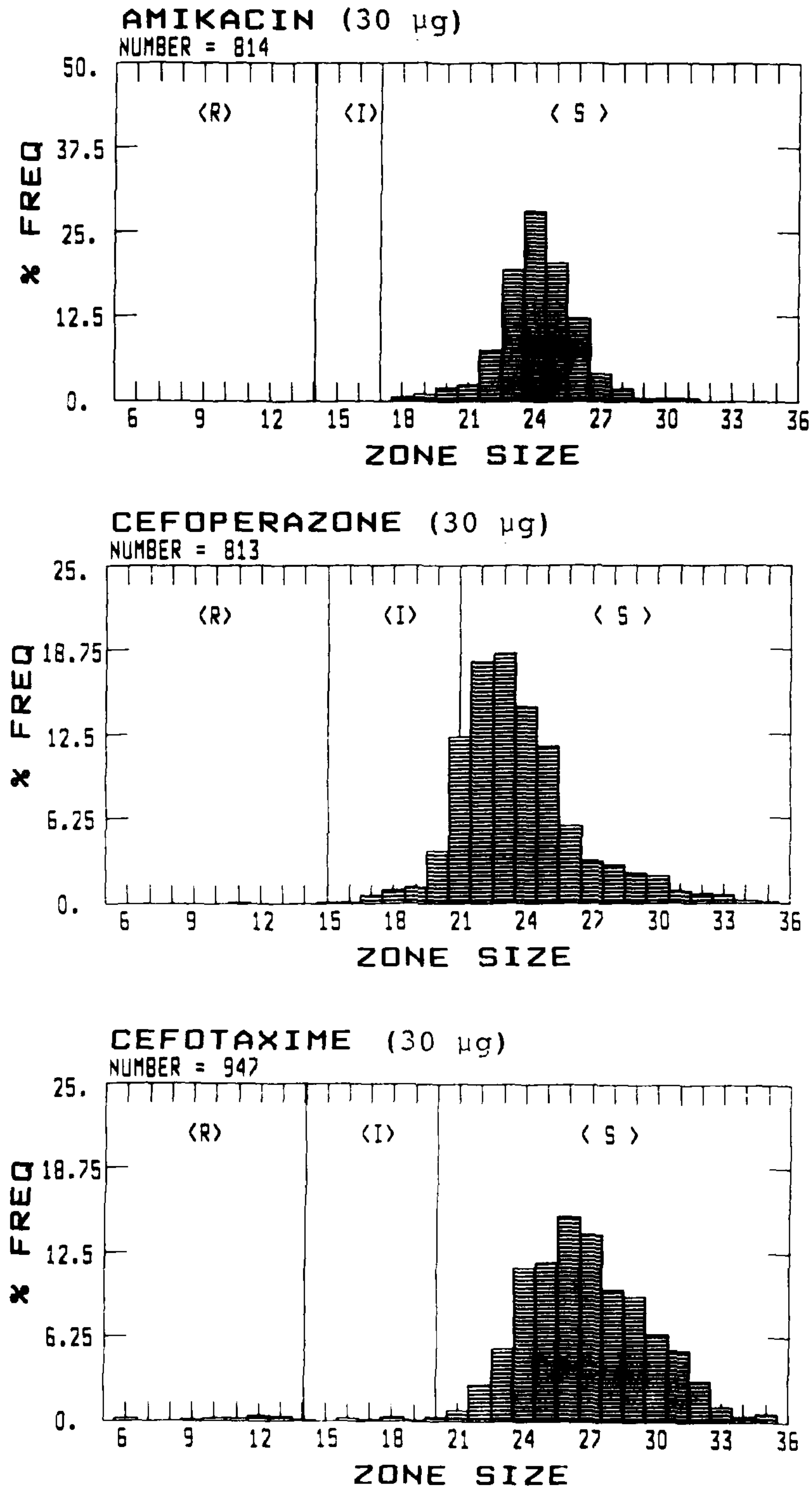


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive Staphylococcus aureus.

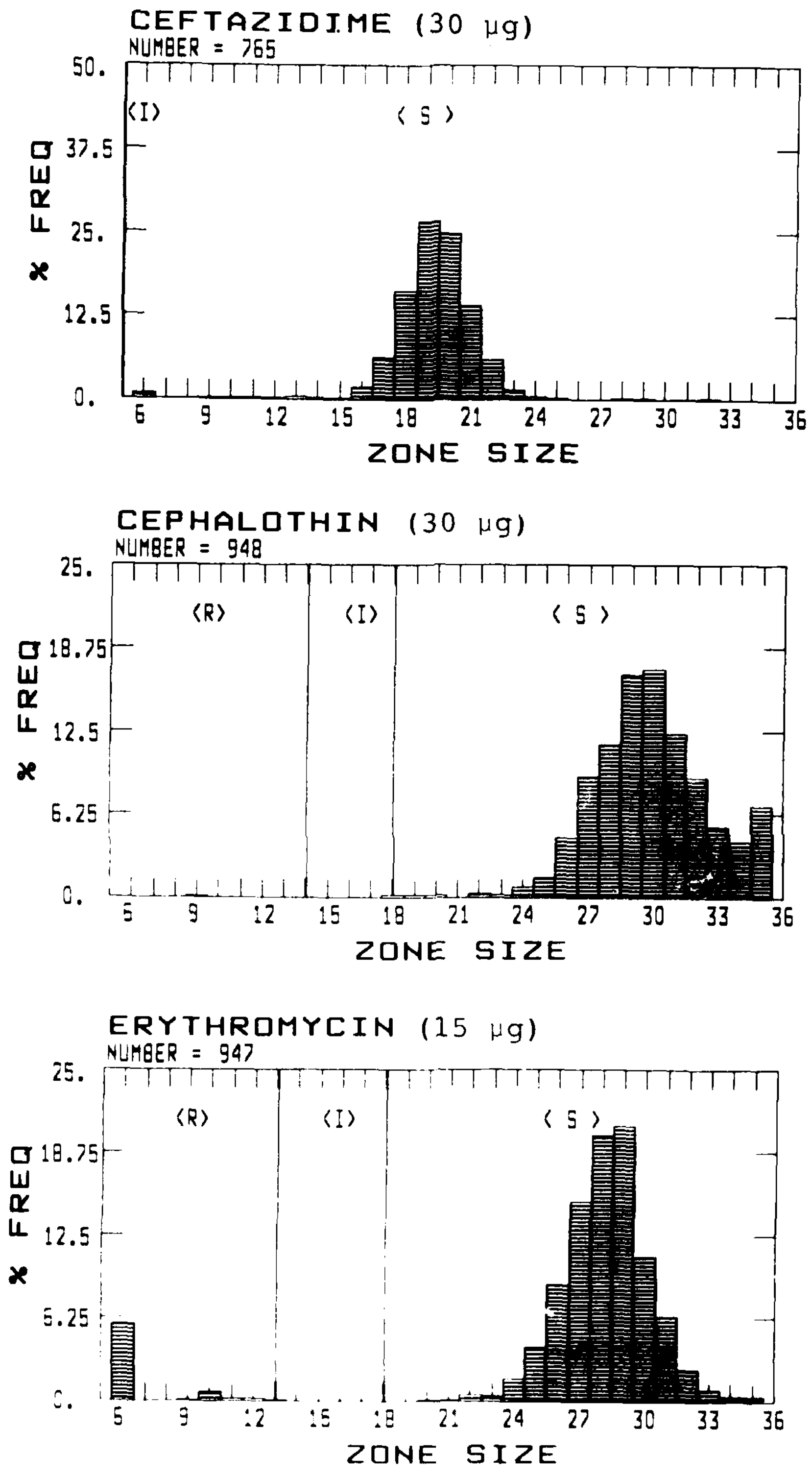


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive <u>Staphylococcus aureus</u> (continued).

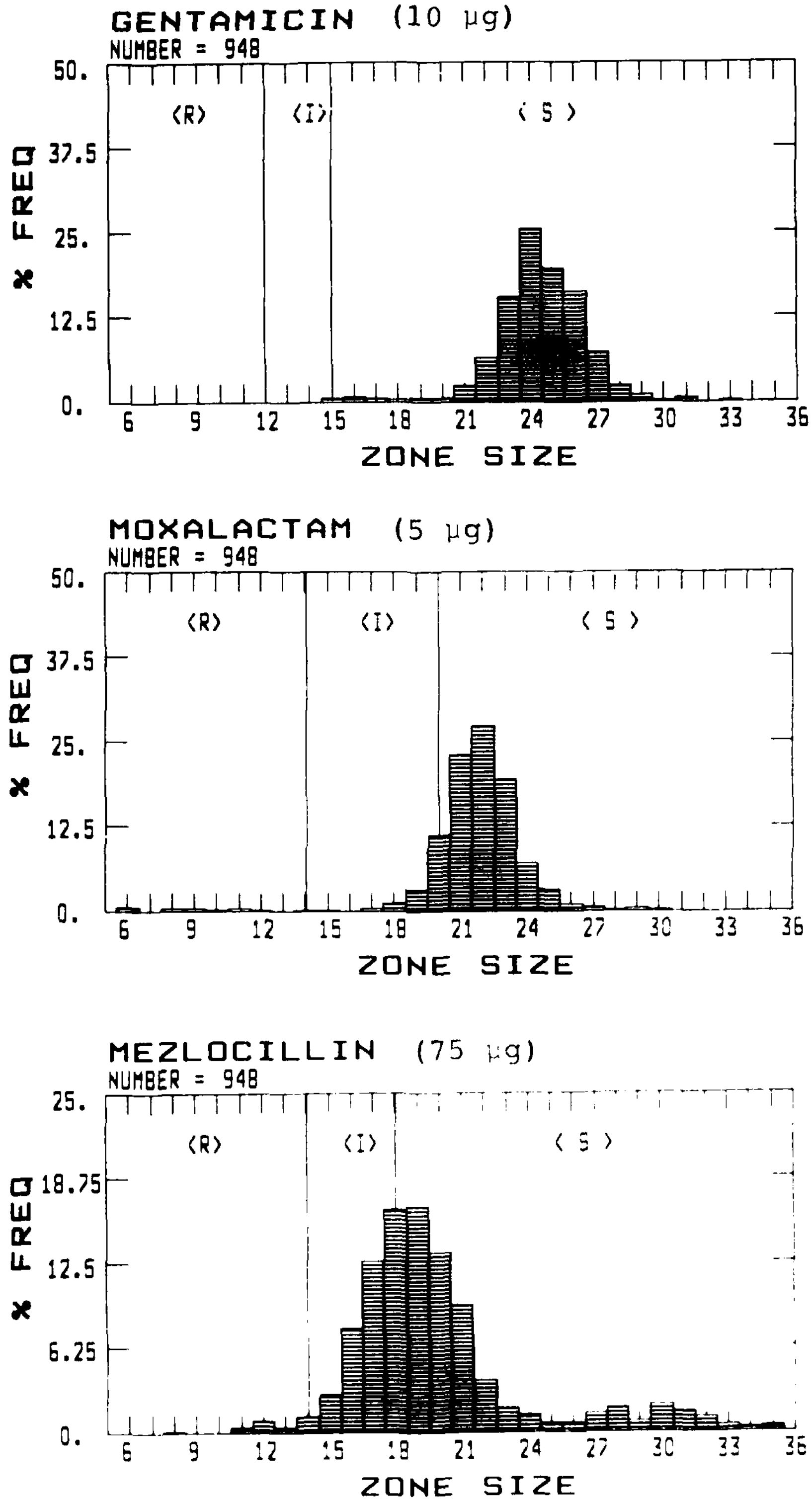


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive Staphylococcus aureus (continued).

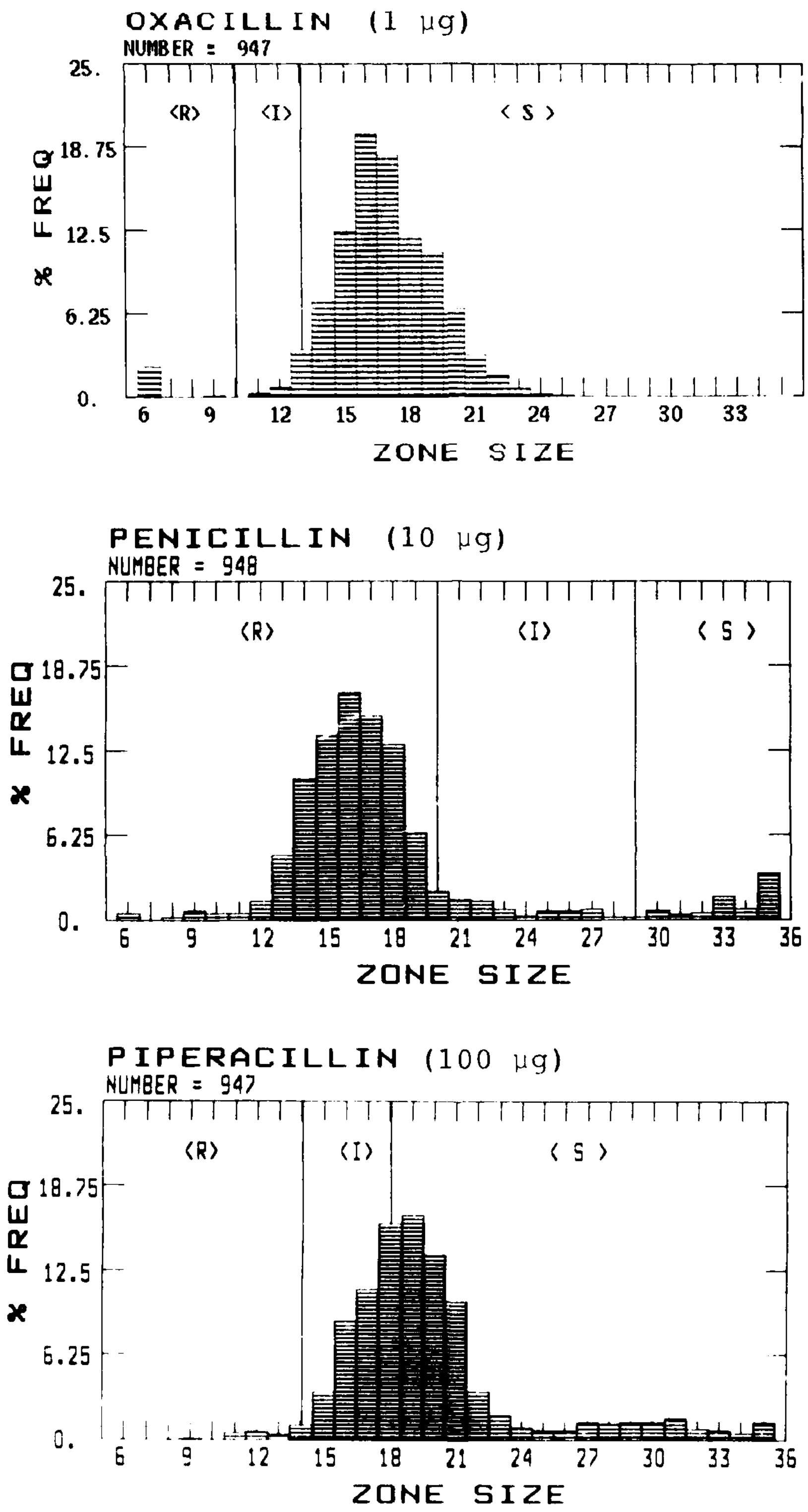


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive Staphylococcus aureus (continued).

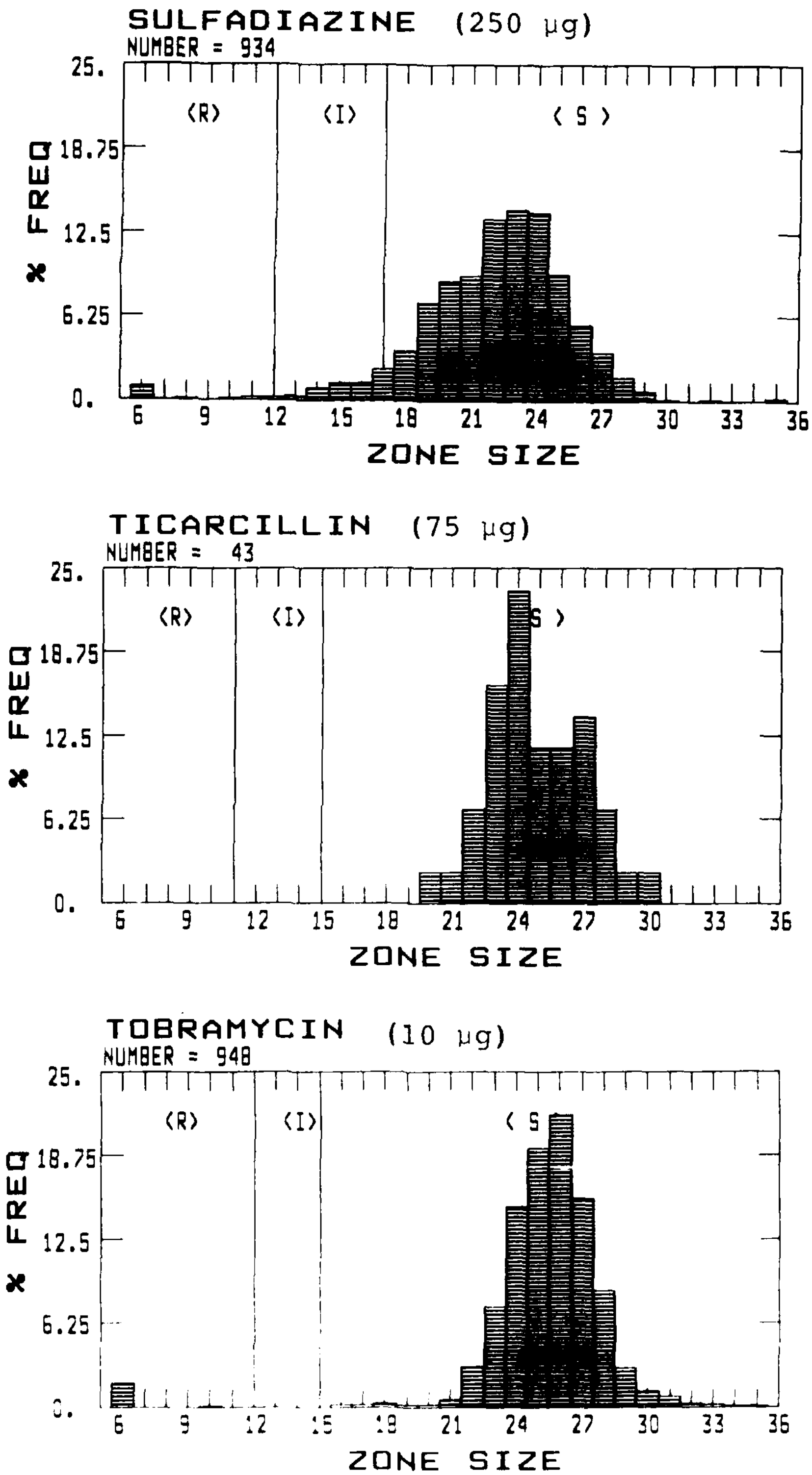


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive <u>Staphylococcus aureus</u> (continued).

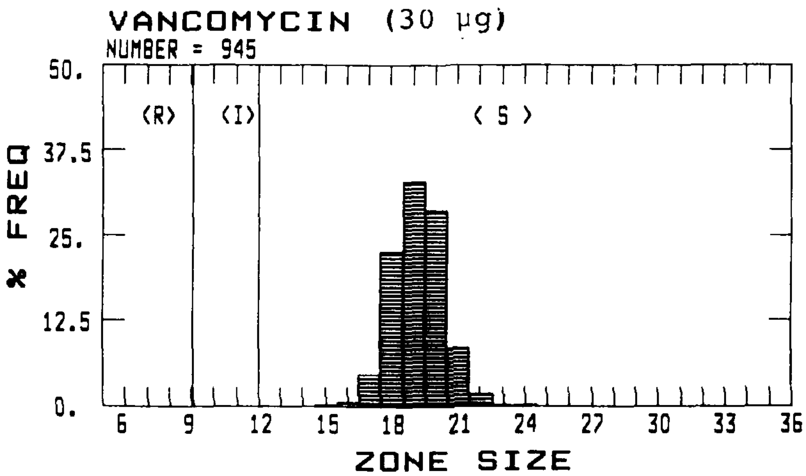


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive Staphylococcus aureus (continued).

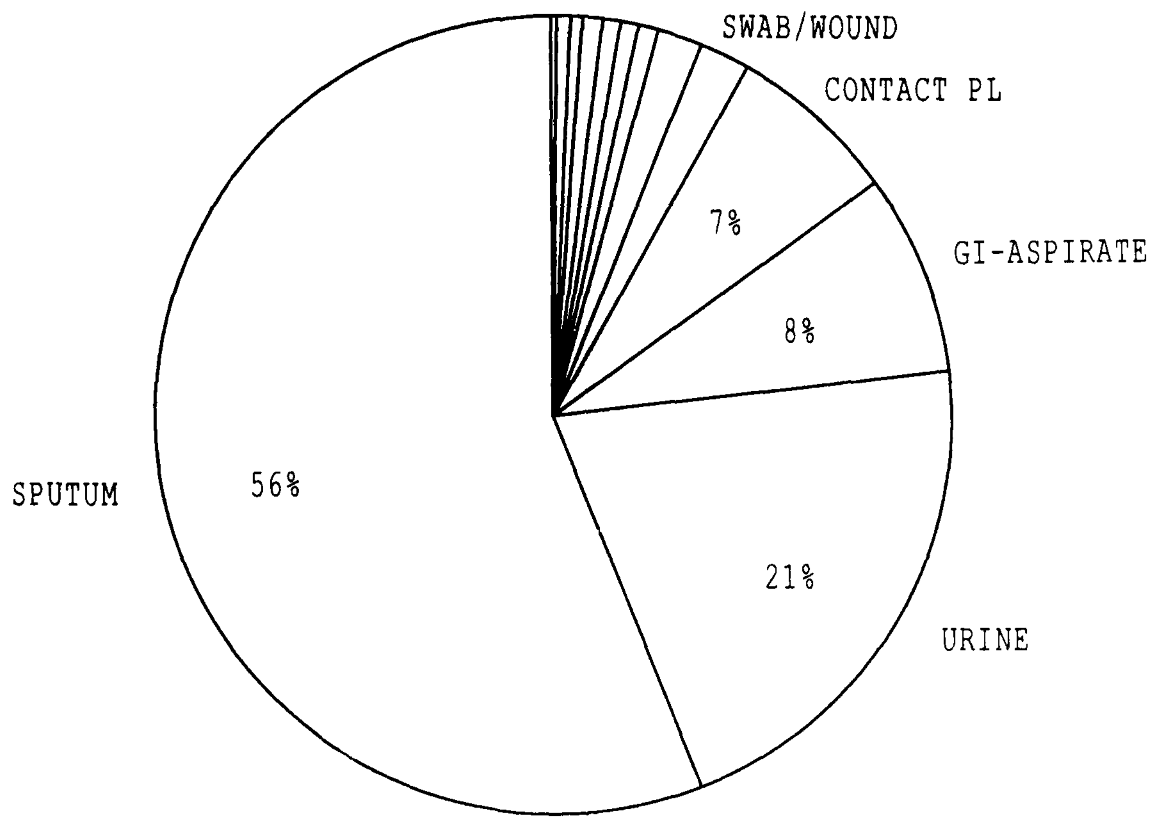


FIGURE 10. Display of the relative frequency of sources yielding *Pseudomonas aeruginosa* tested for *in vitro* sensitivity to antibiotics in 1987.

TABLE 11. Antibiotic Sensitivity Data for *Pseudomonas aeruginosa* (1987)

Antibiotic	RESISTANT %	RESISTANT Number	INTERMEDIATE %	INTERMEDIATE Number	SENSITIVE %	SENSITIVE Number	Total Number
Amikacin	11.33	35	1.62	5	87.06	269	309
Azlocillin	20.68	73	1.42	5	77.90	275	353
Aztreonam	11.67	35	15.33	46	73.00	219	300
Cefoperazone	14.01	43	8.79	27	77.20	237	307
Cefotaxime	28.53	101	64.41	228	7.06	25	354
Cefsulodin	13.97	19	0.00	0	86.03	117	136
Ceftazidime	0.00	0	0.00	0	100.00	290	290
Ceftriaxone	0.00	0	0.00	0	100.00	291	291
Chloramphenicol	77.12	273	20.90	74	1.98	7	354
Colistin	0.32	1	0.65	2	99.03	305	308
Gentamicin	12.99	46	0.85	3	86.16	305	354
Imipenem-cilastatin sodium	5.18	16	0.97	3	93.85	290	309
Kanamycin	96.61	342	1.98	7	1.41	5	354
Mezlocillin	21.75	77	16.67	59	61.58	218	354
Moxalactam	23.51	83	52.97	187	23.51	83	353
Netilmicin	20.06	71	1.41	5	78.53	278	354
Norfloxacin	0.56	2	0.56	2	98.87	350	354
Piperacillin	15.25	54	5.93	21	78.81	279	354
Sulfadiazine	23.78	83	8.02	28	68.19	238	349
Tetracycline	76.27	270	22.60	80	1.13	4	354
Ticarcillin	17.51	62	3.39	12	79.10	280	354
Tobramycin	12.46	44	7.93	28	79.60	281	353
TIM-85	19.46	65	1.50	5	79.04	264	334

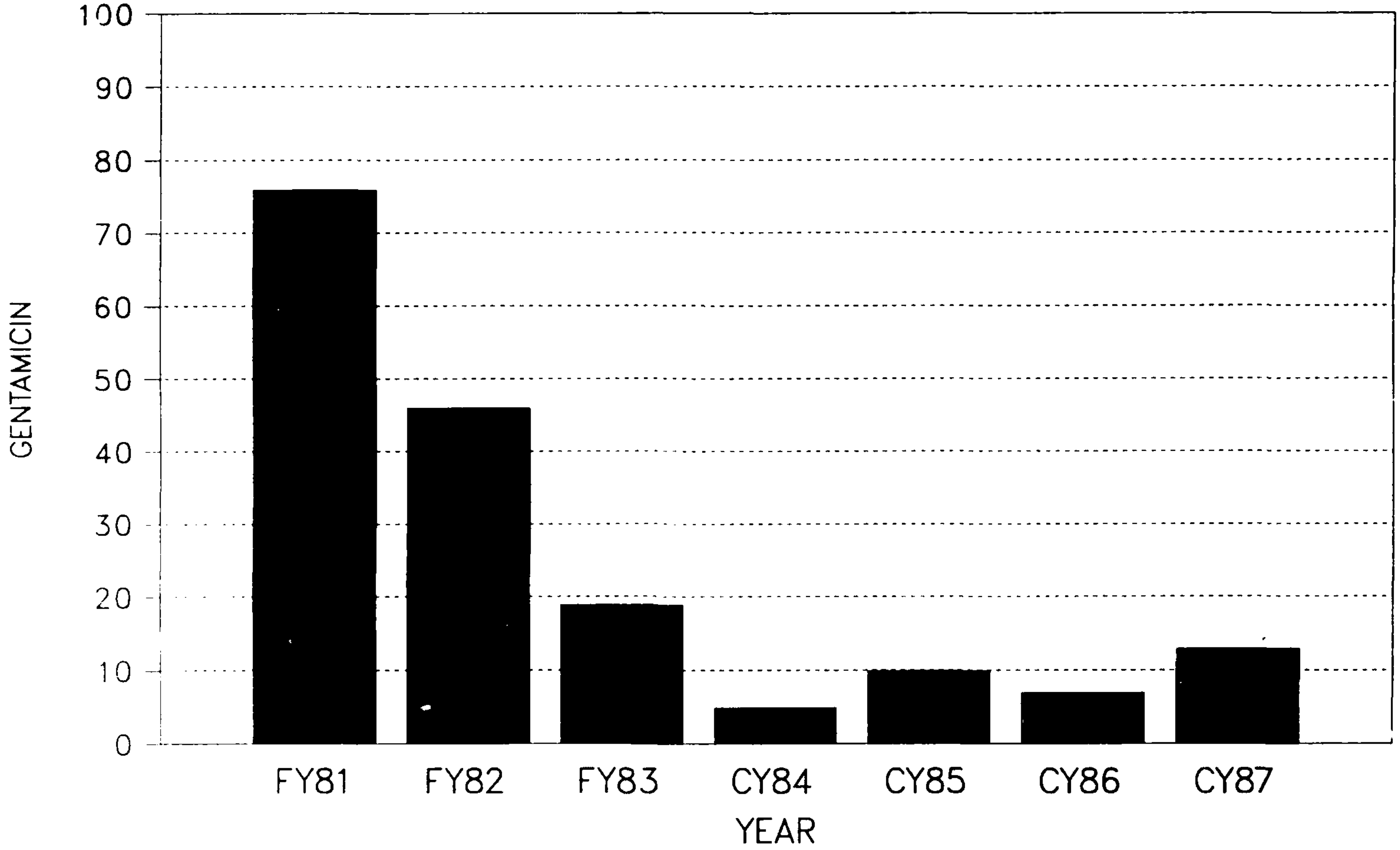


FIGURE 11. Relative frequency (%) of _Pseudomonas aeruginosa_ resistant to gentamicin for fiscal years 1981-4 and calendar years 1935-7.

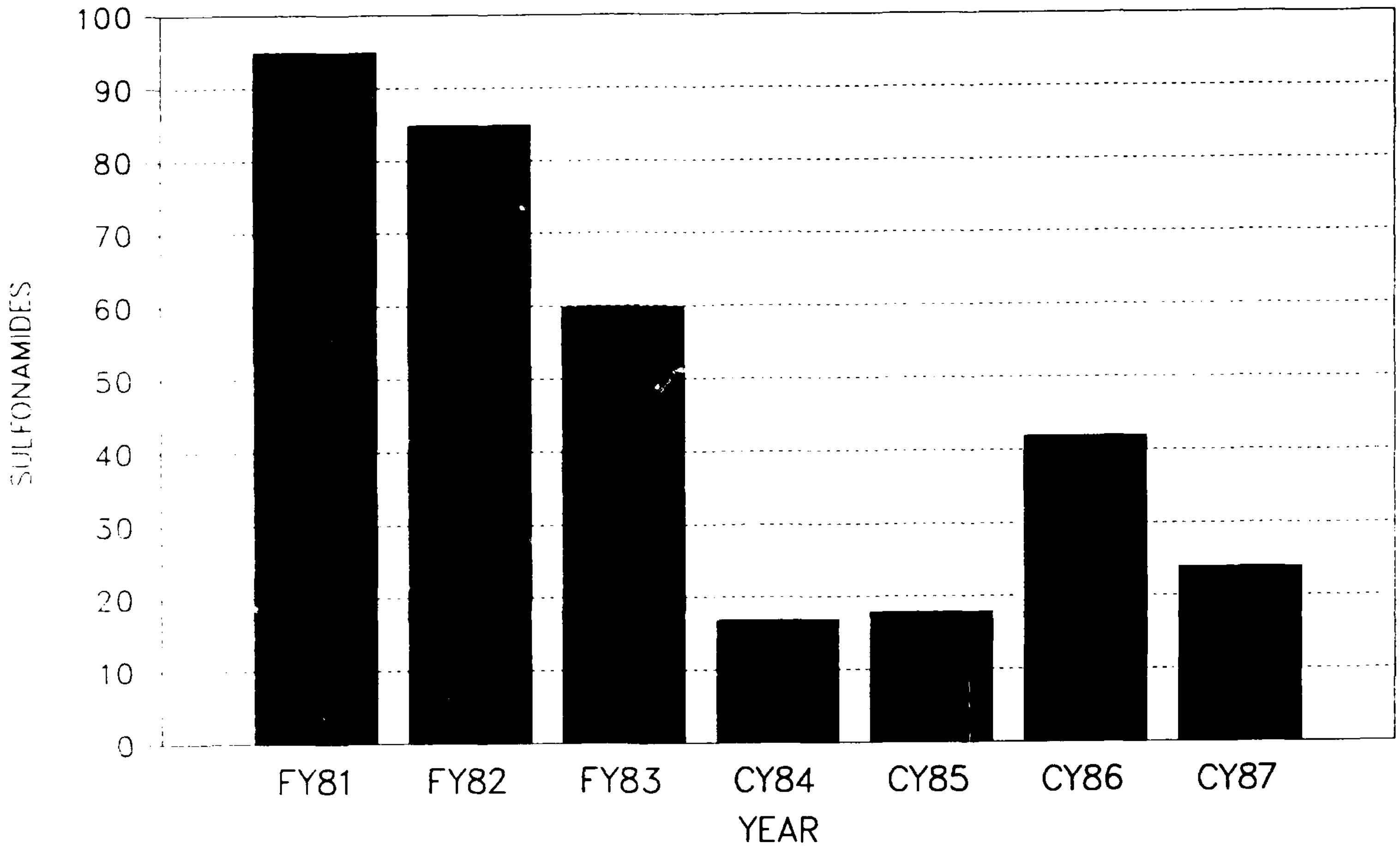


FIGURE 12. Relative frequency (%) of *Pseudomonas aeruginosa* resistance to sulfonamides for fiscal years 1981-4 and calendar years 1985-7.

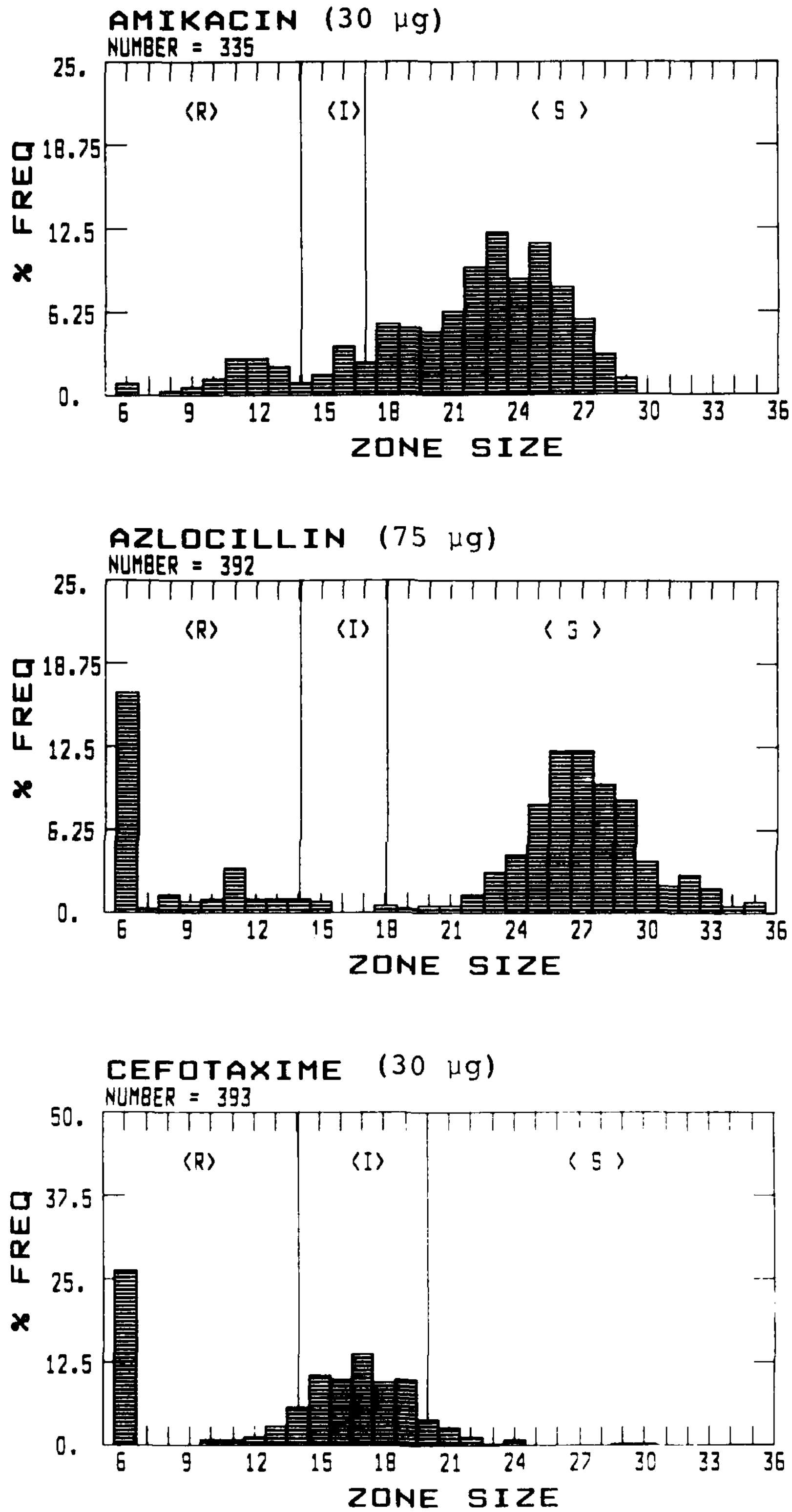


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa.

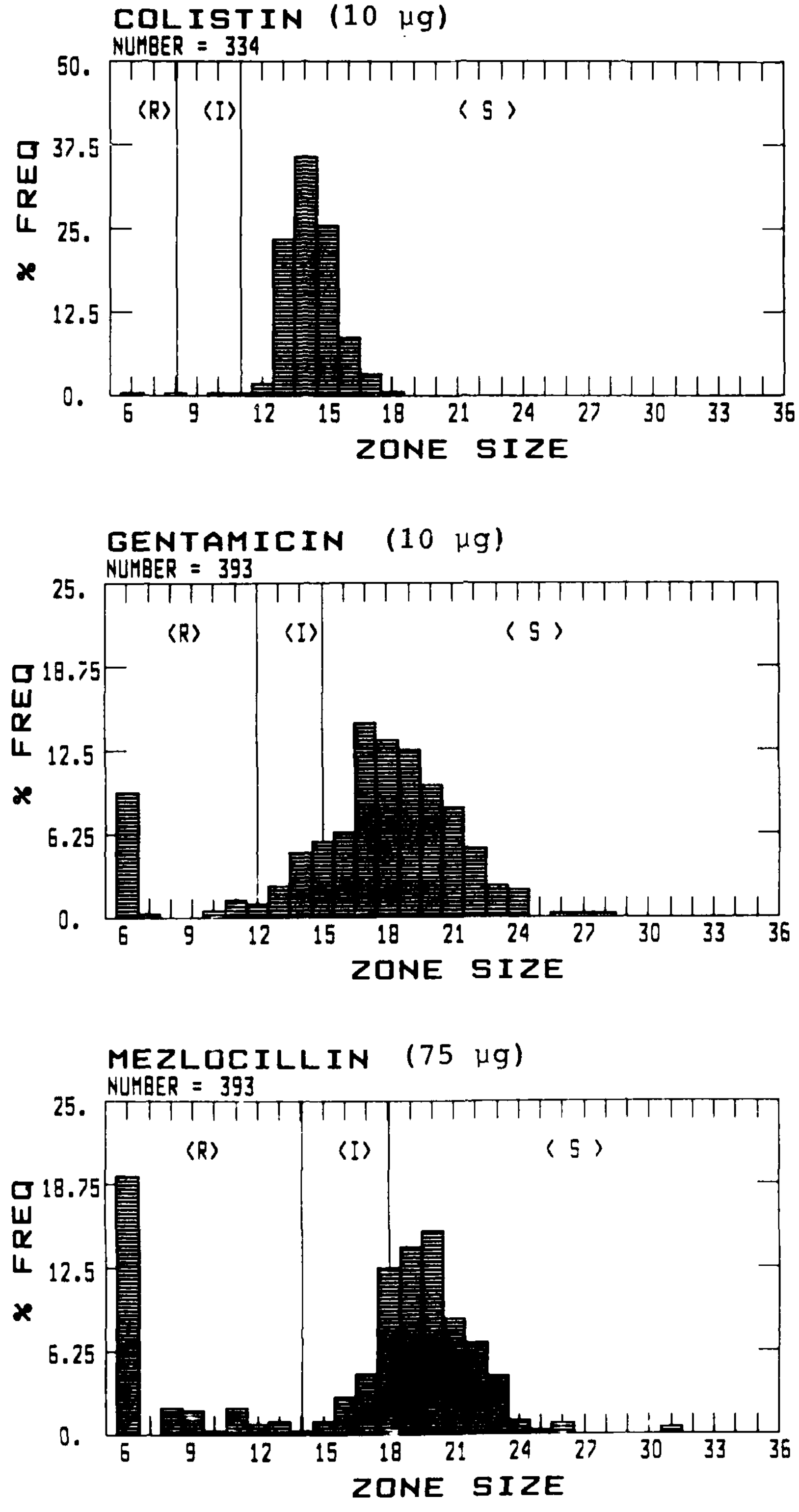


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of <u>Pseudomonas aeruginosa</u> (continued).

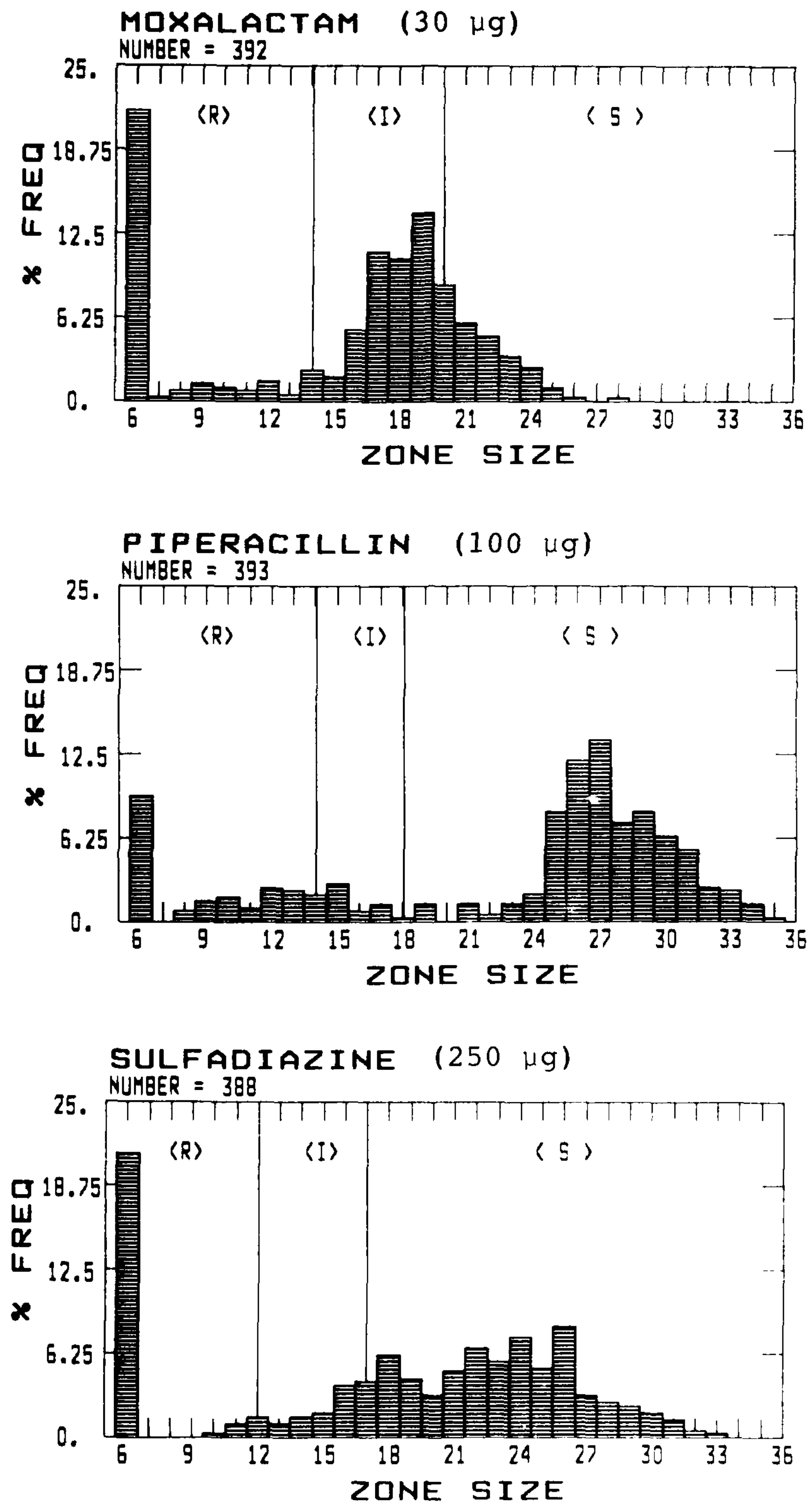
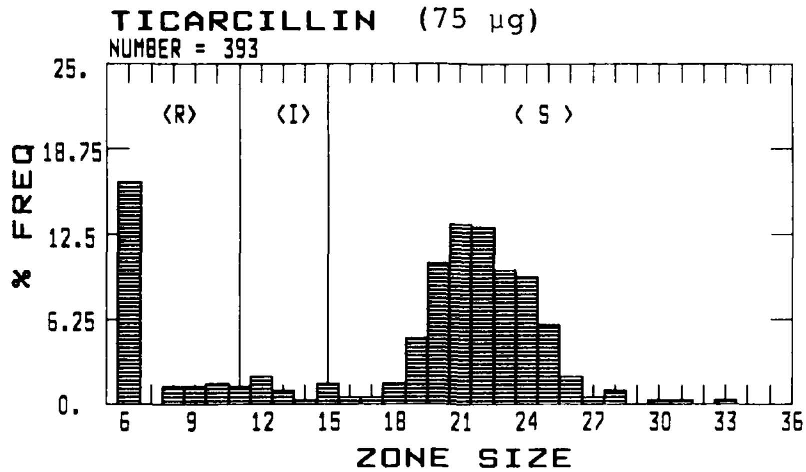


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa (continued).



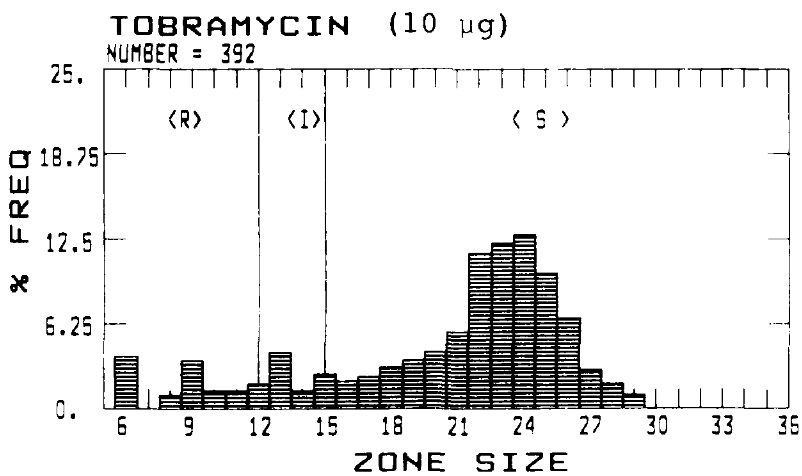


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa (continued).

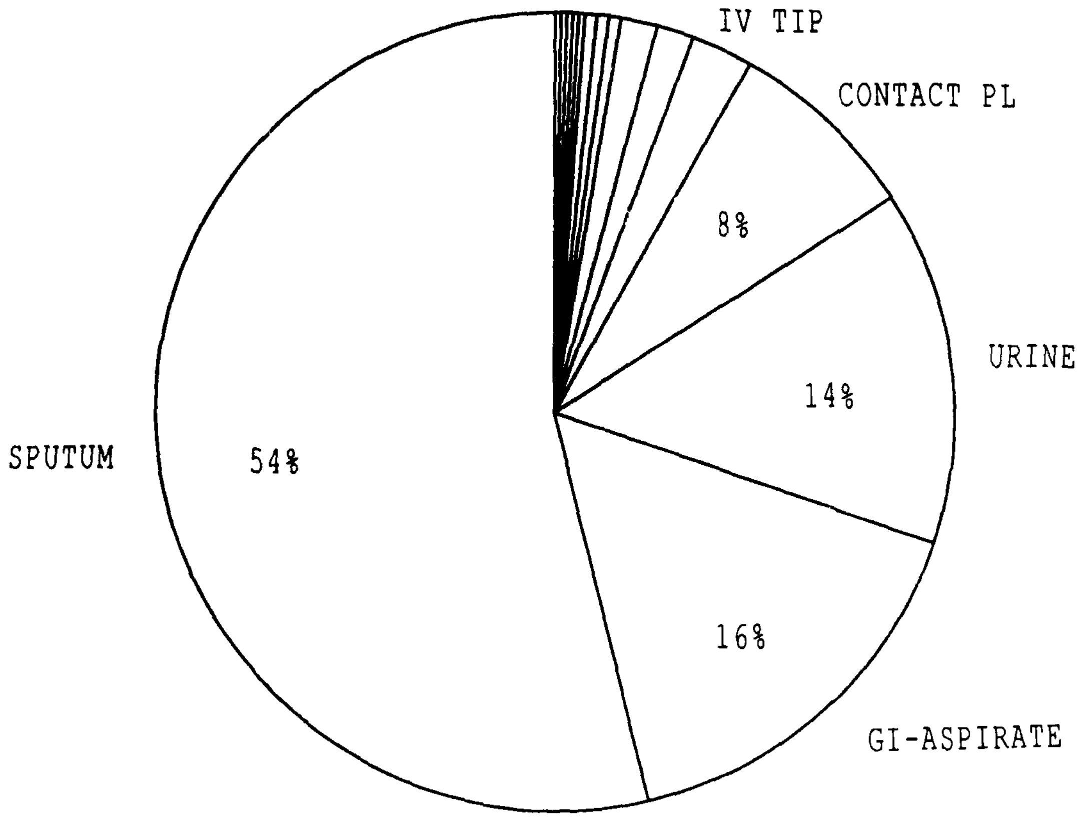


FIGURE 14. Display of the relative frequency of sources yielding *Klebsiella pneumoniae* tested for *in vitro* sensitivity to antibiotics in 1987.

TABLE 12. Antibiotic Sensitivity Data for Klebsiella pneumoniae (1987)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	0.32	1	0.63	2	99.05	313	316
Ampicillin	81.91	335	12.96	53	5.13	21	409
Aztreonam	0.32	1	0.63	2	99.05	314	317
Cefamandole	0.49	2	2.20	9	97.31	398	409
Cefoperazone	0.00	0	2.86	9	97.14	306	315
Cefotaxime	0.24	1	0.24	1	99.51	407	409
Cefoxitin	1.47	6	1.47	6	97.07	397	409
Ceftazidime	0.00	0	0.00	0	100.00	280	280
Ceftriaxone	0.00	0	0.00	0	100.00	280	280
Chloramphenicol	3.91	16	0.73	3	95.35	390	409
Gentamicin	0.24	1	1.96	8	97.80	400	409
Imipenem-cilastatin sodium	0.00	0	0.00	0	100.00	316	316
Kanamycin	1.72	7	6.37	26	91.91	375	408
Mezlocillin	2.69	11	13.94	57	83.37	341	409
Moxalactam	0.00	0	0.00	0	100.00	33	33
Nalidixic acid	0.49	2	1.96	8	97.56	399	409
Netilimicin	0.00	0	0.25	1	99.75	407	408
Norfloxacin	0.00	0	0.00	0	100.00	408	408
Piperacillin	1.96	8	5.87	24	92.18	377	409
Streptomycin	12.66	40	24.37	77	62.97	199	316
Sulfadiazine	66.26	271	0.49	2	33.25	136	409
Tetracycline	8.80	36	3.67	15	87.53	358	409
Ticarcillin	69.44	284	22.98	94	7.58	31	409
Tobramycin	0.00	0	0.00	0	100.00	33	33
Trimethoprim	3.91	16	1.71	7	94.38	386	409
Trimeth & Sulfa	6.86	28	3.43	14	89.71	366	408

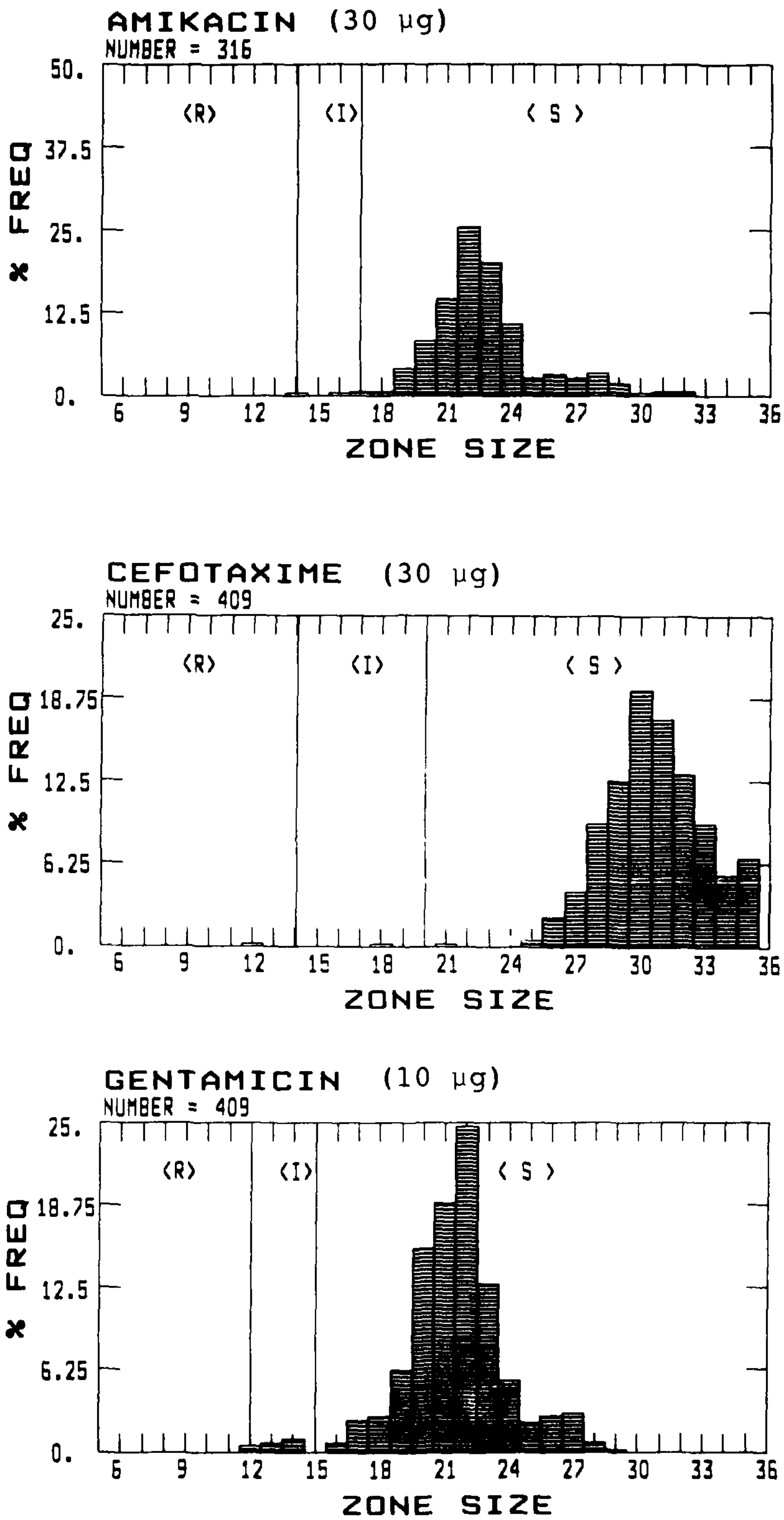


FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae*.

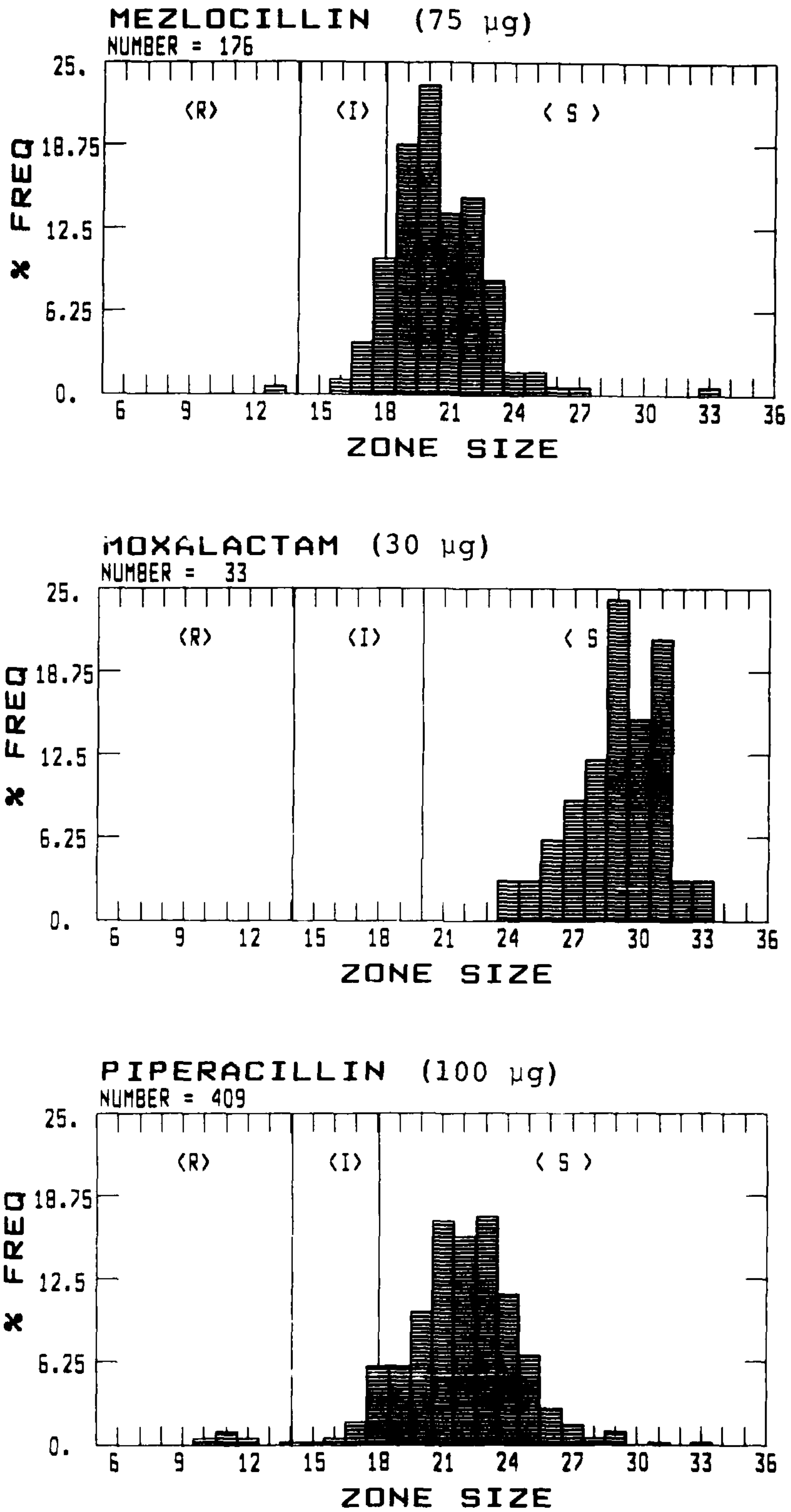


FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of Klebsiella pneumoniae (continued).

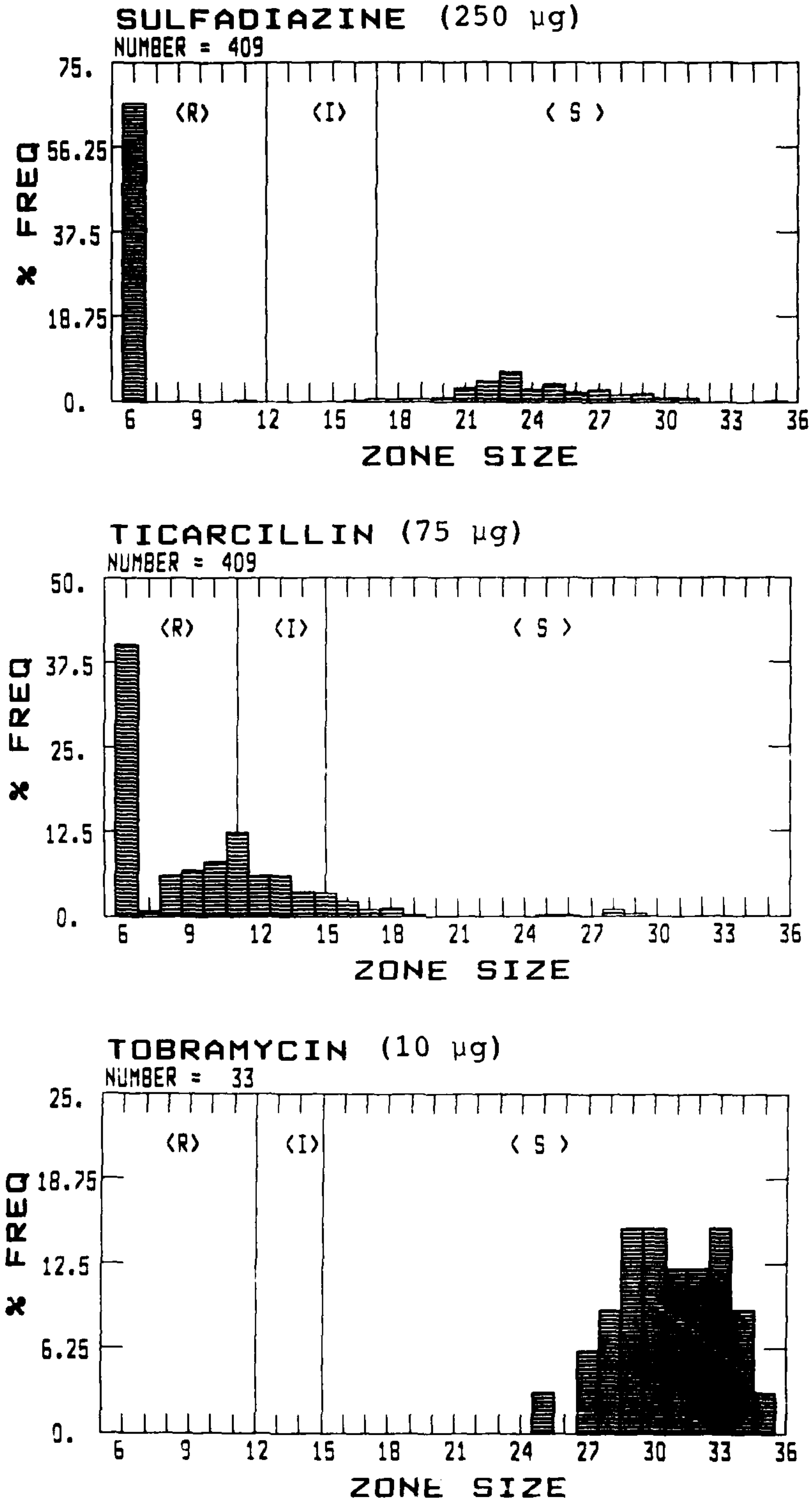


FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae* (continued).

Enterobacter aerogenes. The sources of isolation for tested strains are presented in Figure 16. The results of *in vitro* antibiotic testing are presented in Table 13. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 17.

Enterobacter cloacae. The sources of isolation for tested strains are presented in Figure 18. The results of *in vitro* antibiotic testing are presented in Table 14. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 19.

Escherichia coli. The sources of isolation for tested strains are presented in Figure 20. The results of *in vitro* antibiotic testing are presented in Table 15. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 21.

PRESENTATIONS/PUBLICATIONS

McManus AT: The rise and fall of Pseudomonas. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 October 1987.

McManus AT: Occurrence of *Pseudomonas aeruginosa* in seriously burned patients: A review of 950 patients (1983-1987). Presented at the International Symposium on Basic Research and Clinical Aspects of *Pseudomonas aeruginosa* infections, Copenhagen, Denmark, 1 September 1988.

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1. McManus AT, Henderson JR, Lawson TJ, *et al*: Studies of Infection and Microbiologic Surveillance of Infection in Troops with Thermal Injury. In **US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1985**, c1987, pp 146-194.

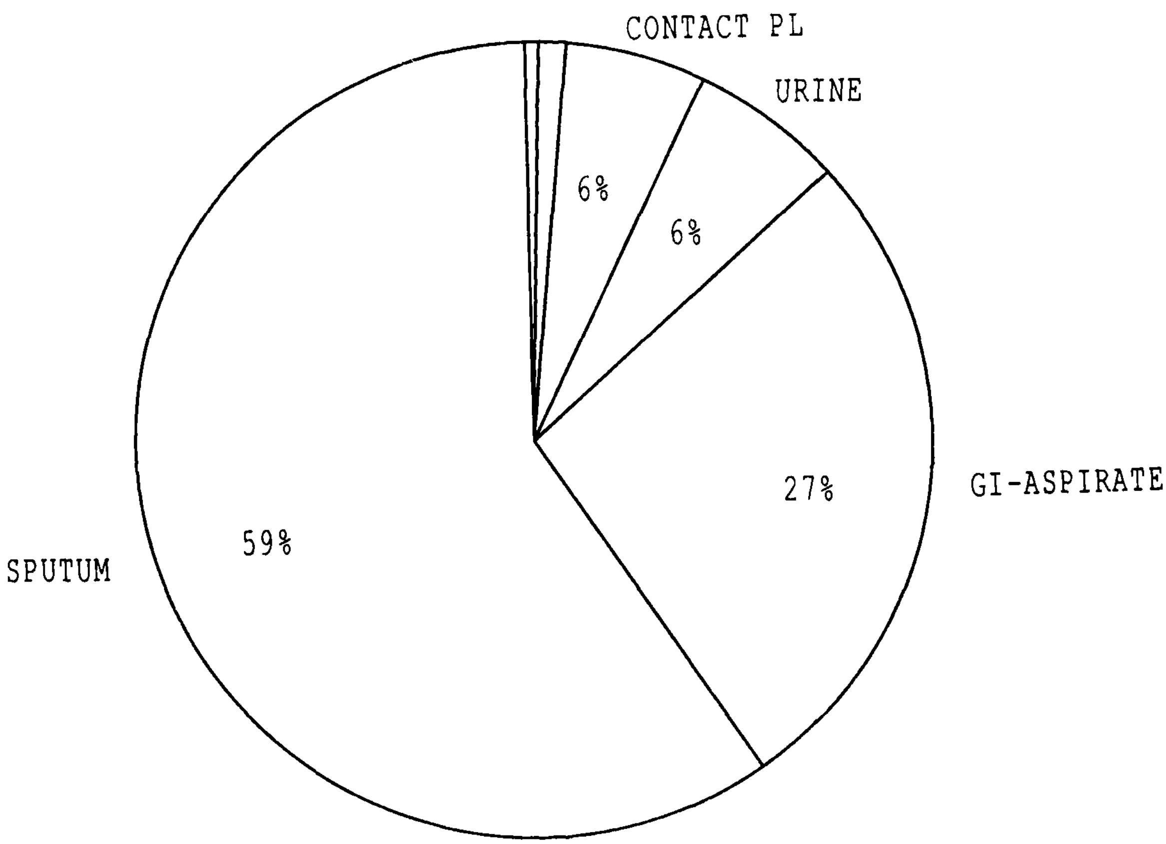


FIGURE 16. Display of the relative frequency of sources yielding Enterobacter aerogenes tested for in vitro sensitivity to antibiotics in 1987.

TABLE 13. Antibiotic Sensitivity Data for <u>Enterobacter aerogenes</u> (1987)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	0.00	0	1.32	2	98.68	149	151
Ampicillin	91.48	161	0.57	1	7.95	14	176
Aztreonam	24.64	34	5.80	8	69.57	96	138
Cefamandole	42.05	74	6.82	12	51.14	90	176
Cefoperazone	3.31	5	32.45	49	64.24	97	151
Cefotaxime	30.68	54	2.84	5	66.48	117	176
Cefoxitin	83.52	147	2.84	5	13.64	24	176
Ceftazidime	0.00	0	0.00	0	100.00	132	132
Ceftriaxone	0.00	0	0.00	0	100.00	132	132
Chloramphenicol	3.98	7	6.82	12	89.20	157	157
Gentamicin	0.57	1	1.14	2	98.30	173	176
Imipenem-cilastatin sodium	0.66	1	0.00	0	99.34	150	151
Kanamycin	3.98	7	7.39	13	88.64	156	176
Mezlocillin	23.86	42	13.64	24	62.50	110	176
Moxalactam	47.37	9	0.00	0	52.63	10	19
Nalidixic acid	0.00	0	6.25	11	93.75	165	176
Netilimicin	0.00	0	0.57	1	99.43	175	176
Norfloxacin	0.00	0	0.00	0	100.00	176	176
Piperacillin	27.27	48	9.66	17	63.07	111	176
Streptomycin	1.99	3	5.96	9	92.05	139	151
Sulfadiazine	12.50	22	3.98	7	83.52	147	176
Tetracycline	9.09	16	14.77	26	76.14	134	176
Ticarcillin	33.52	59	4.55	8	61.93	109	176
Tobramycin	42.11	8	5.26	1	52.63	10	19
Trimethoprim	2.84	5	2.84	5	94.32	166	176
Trimeth & Sulfa	3.43	6	0.00	0	96.57	169	175

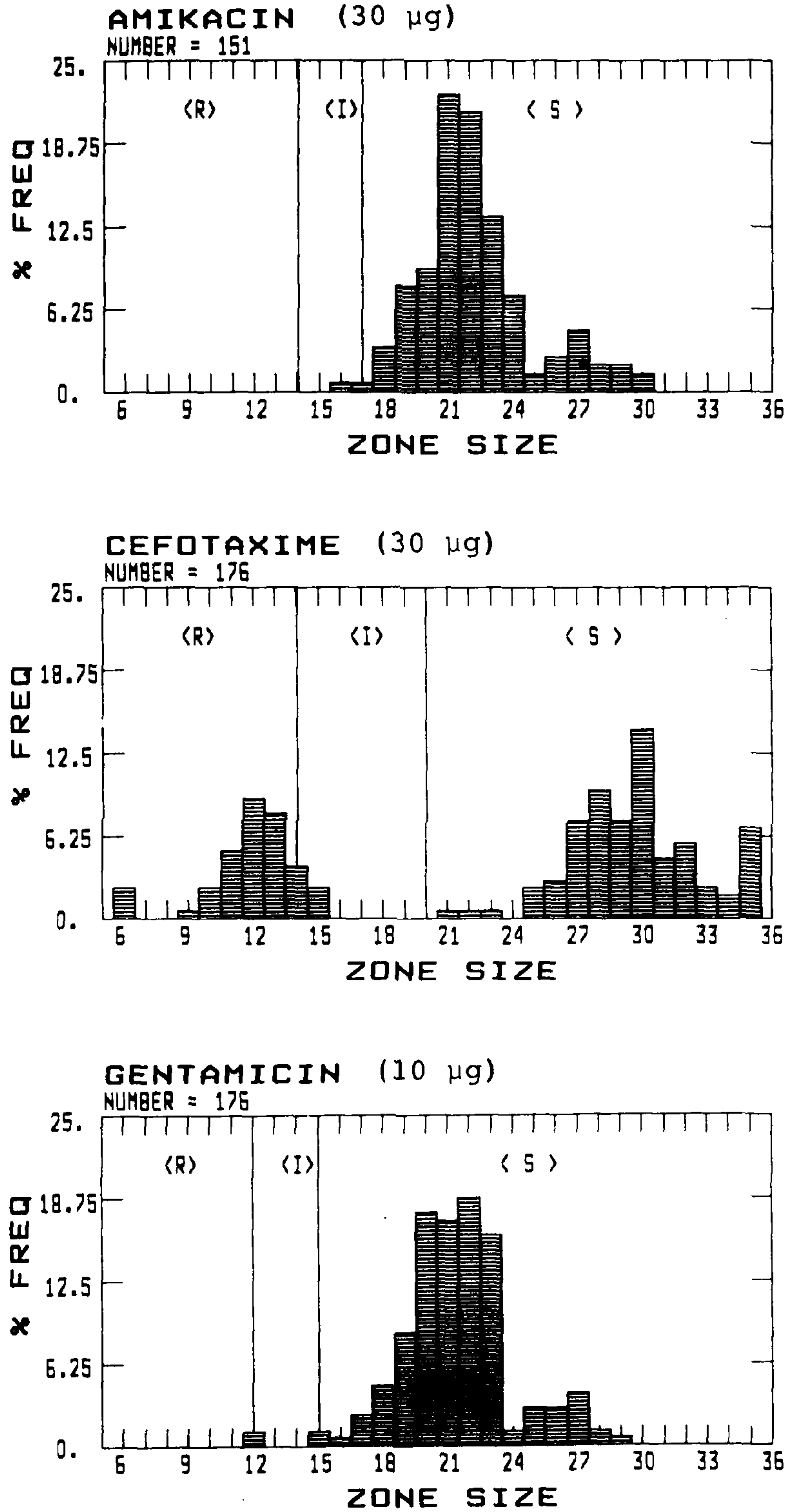
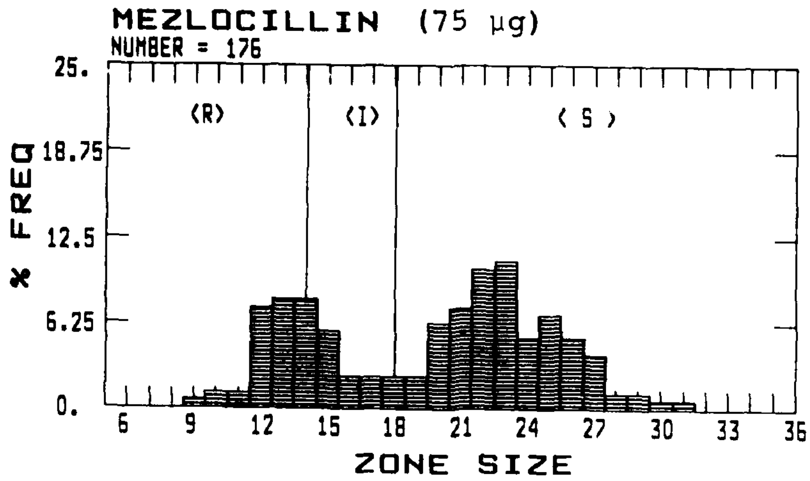
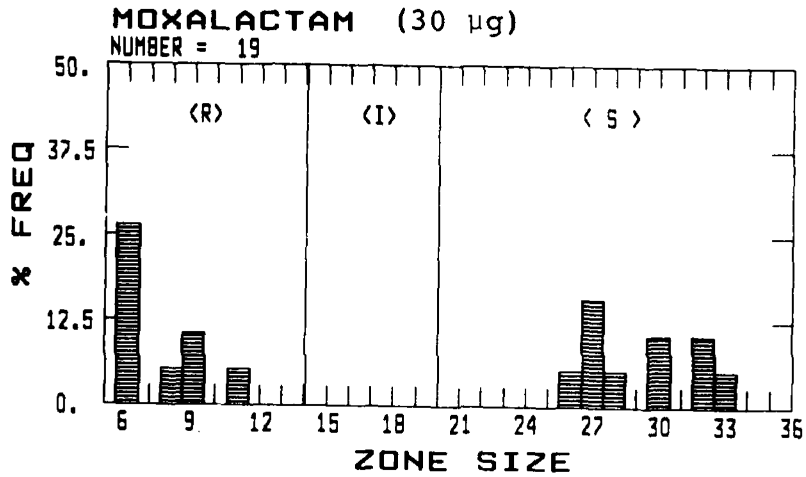


FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of Enterobacter aerogenes.





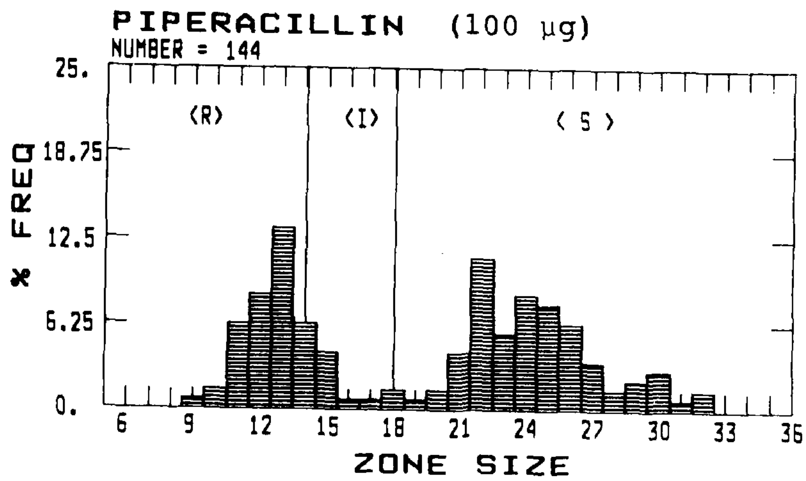


FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of <u>Enterobacter aerogenes</u> (continued).

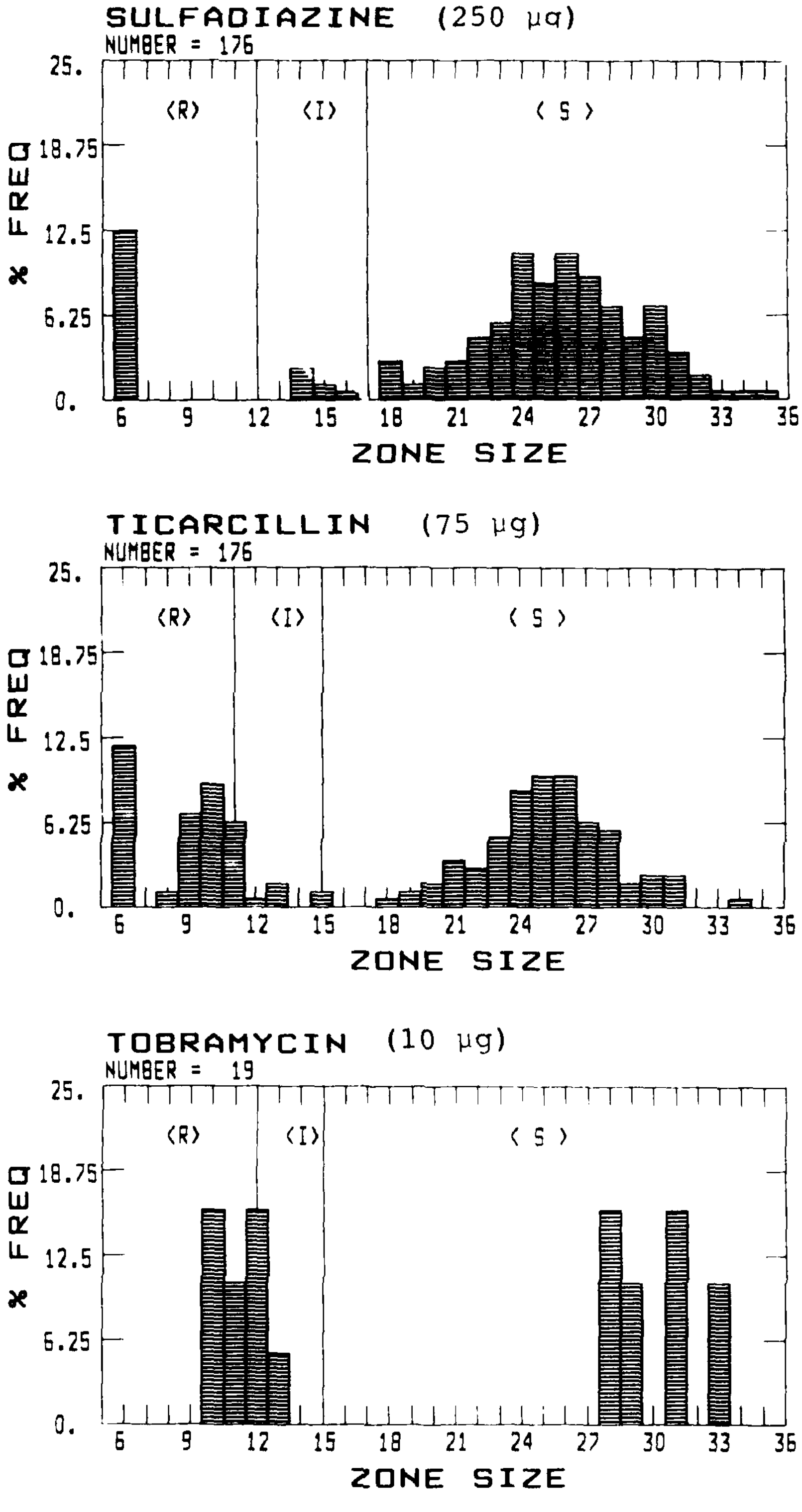


FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of *Enterobacter aerogenes* (continued).

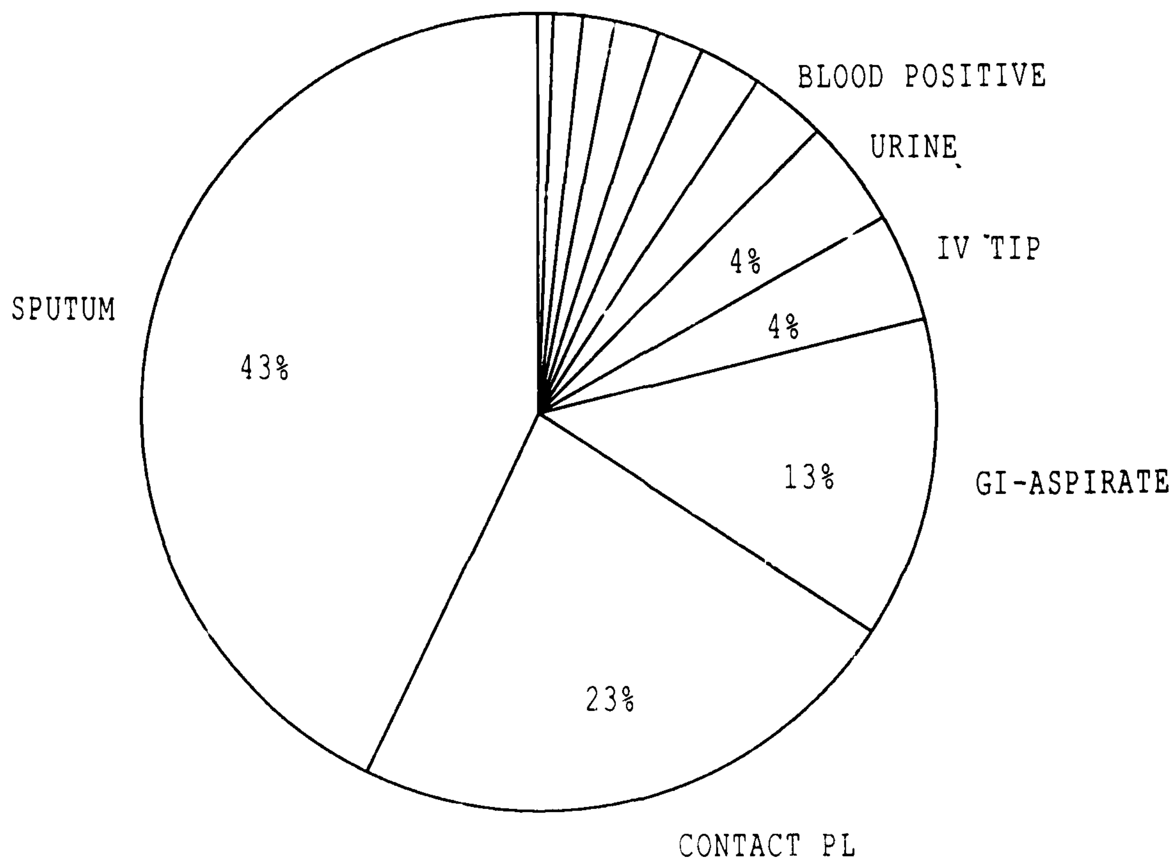


FIGURE 18. Display of the relative frequency of sources yielding Enterobacter cloacae tested for in vitro sensitivity to antibiotics in 1987.

TABLE 14. Antibiotic Sensitivity Data for Enterobacter cloacae (1987)

Antibiotic	RESISTANT %	RESISTANT Number	INTERMEDIATE %	INTERMEDIATE Number	SENSITIVE %	SENSITIVE Number	Total Number
Amikacin	0.00	0	4.76	7	95.24	140	147
Ampicillin	83.66	128	5.88	9	10.46	16	153
Aztreonam	22.45	33	11.56	17	65.99	97	147
Cefamandole	39.87	61	1.31	2	58.82	90	153
Cefoperazone	15.65	23	17.69	26	66.67	98	147
Cefotaxime	31.17	48	0.65	1	68.18	105	154
Cefoxitin	88.89	136	0.00	0	11.11	17	153
Ceftazidime	0.00	0	0.00	0	100.00	133	133
Ceftriaxone	0.00	0	0.00	0	100.00	136	136
Chloramphenicol	6.49	10	11.69	18	81.82	126	154
Gentamicin	0.65	1	1.30	2	98.05	151	154
Imipenem-cilastatin sodium	0.68	1	0.00	0	99.32	146	147
Kanamycin	5.19	8	5.19	8	89.61	138	154
Mezlocillin	3.25	5	24.03	37	72.73	112	154
Moxalactam	33.33	3	0.00	0	66.67	6	9
Nalidixic acid	0.00	0	30.07	46	69.93	107	153
Netilimicin	1.30	2	0.65	1	98.05	151	154
Norfloxacin	0.00	0	0.65	1	99.35	153	154
Piperacillin	3.25	5	25.32	39	71.43	110	154
Streptomycin	10.20	15	33.33	49	56.46	83	147
Sulfadiazine	47.40	73	0.00	0	52.60	81	154
Tetracycline	1.95	3	31.17	48	66.88	103	154
Ticarcillin	34.42	53	1.30	2	64.29	99	154
Tobramycin	22.22	2	0.00	0	77.78	7	9
Trimethoprim	5.23	8	0.65	1	94.12	144	153
Trimeth & Sulfa	5.23	8	0.65	1	94.12	144	153

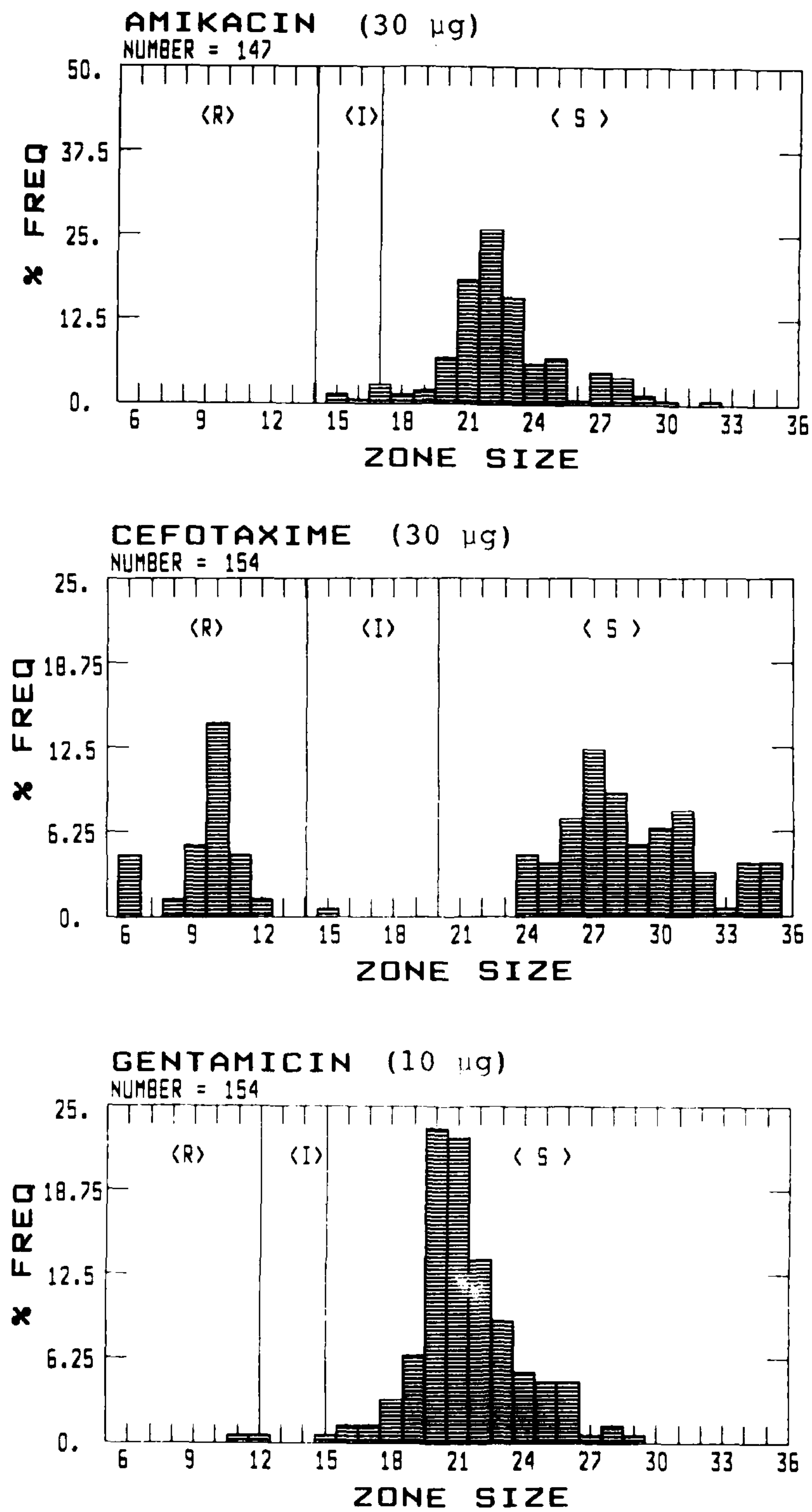
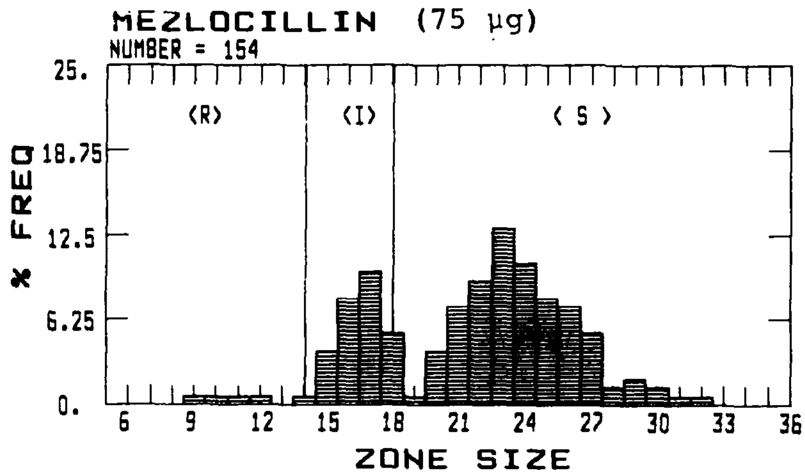
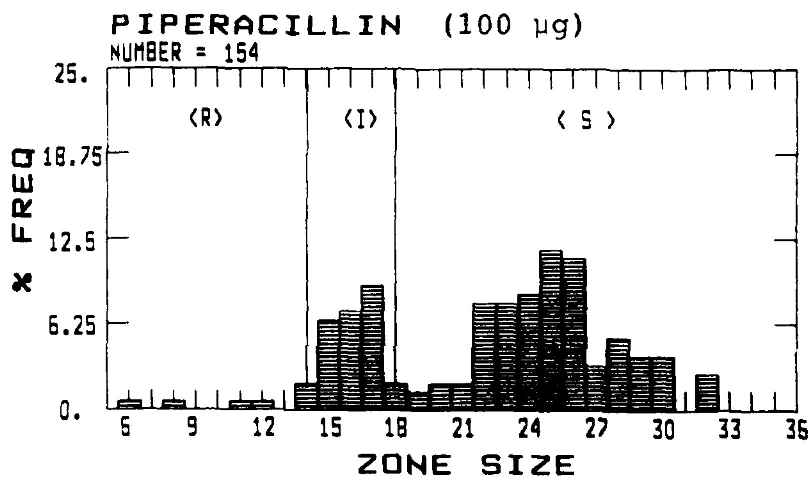


FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of Enterobacter cloacae.





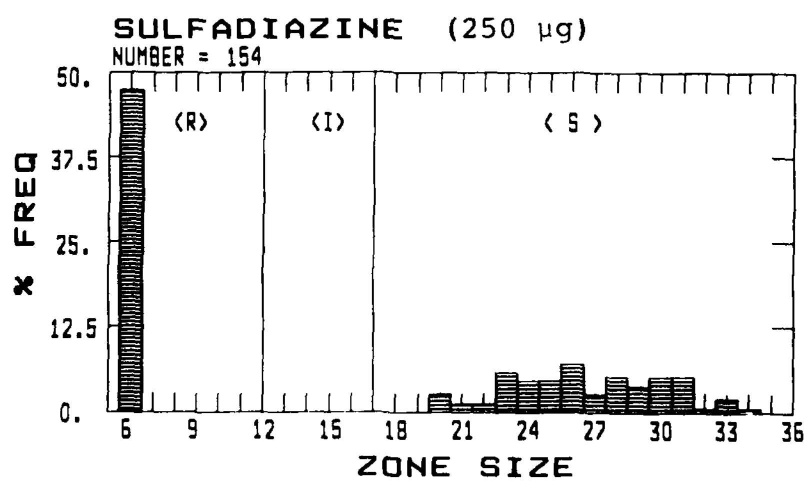


FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of *Enterobacter cloacae* (continued).

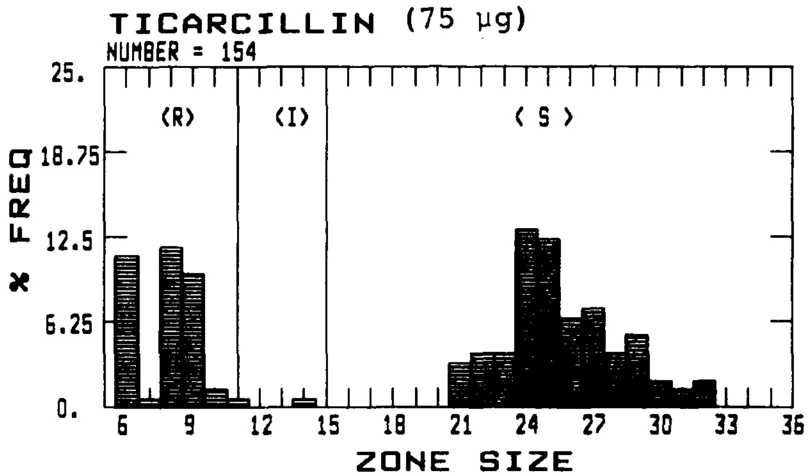


FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of <u>Enterobacter cloacae</u> (continued).

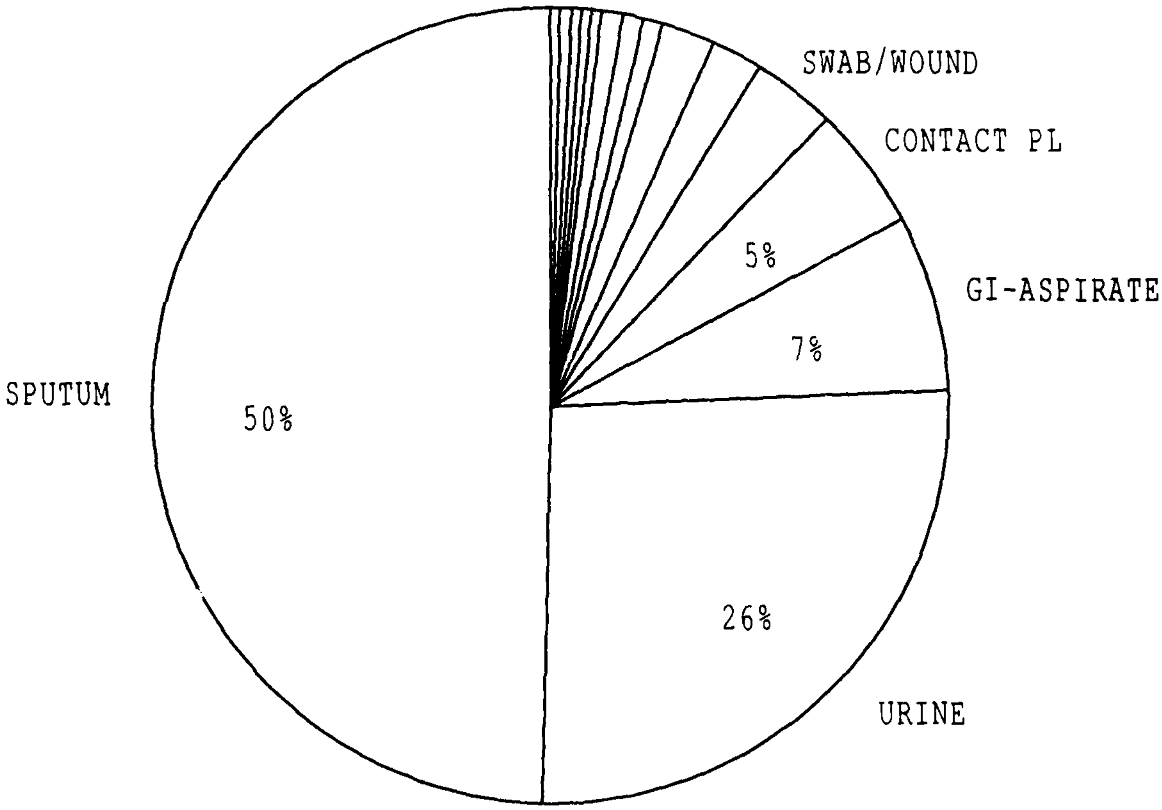


FIGURE 20. Display of the relative frequency of sources yielding *Escherichia coli* tested for *in vitro* sensitivity to antibiotics in 1987.

TABLE 15. Antibiotic Sensitivity Data for _Escherichia coli_ (1987)

Antibiotic	RESISTANT %	RESISTANT Number	INTERMEDIATE %	INTERMEDIATE Number	SENSITIVE %	SENSITIVE Number	Total Number
Amikacin	0.52	1	3.09	6	96.39	187	194
Ampicillin	40.52	94	1.72	4	57.76	134	232
Aztreonam	0.00	0	1.78	3	98.22	166	169
Cefamandole	1.28	3	11.54	27	87.18	204	234
Cefoperazone	1.54	3	13.33	26	85.13	166	195
Cefotaxime	0.00	0	0.43	1	99.57	232	233
Cefoxitin	2.99	7	1.71	4	95.30	223	234
Ceftazidime	0.00	0	0.00	0	100.00	180	180
Ceftriaxone	0.00	0	0.00	0	100.00	182	182
Chloramphenicol	15.38	36	6.84	16	77.78	182	234
Gentamicin	0.43	1	0.43	1	99.15	232	234
Imipenem-cilastatin sodium	0.00	0	0.00	0	100.00	195	195
Kanamycin	15.45	36	3.86	9	80.69	188	233
Mezlocillin	21.37	50	14.96	35	63.68	149	234
Moxalactam	0.00	0	0.00	0	100.00	12	12
Nalidixic acid	0.43	1	1.30	3	98.27	227	231
Netilimicin	0.43	1	0.00	0	99.57	232	233
Norfloxacin	0.44	1	0.00	0	99.56	227	228
Piperacillin	20.09	47	14.96	35	64.96	152	234
Streptomycin	46.91	91	9.79	19	43.30	84	194
Sulfadiazine	64.10	150	0.43	1	35.47	83	234
Tetracycline	36.32	85	3.85	9	59.83	140	234
Ticarcillin	37.34	87	0.43	1	62.23	145	233
Tobramycin	0.00	0	0.00	0	100.00	13	13
Trimethoprim	5.98	14	1.71	4	92.31	216	234
Trimeth & Sulfa	7.30	17	5.15	12	87.55	204	233

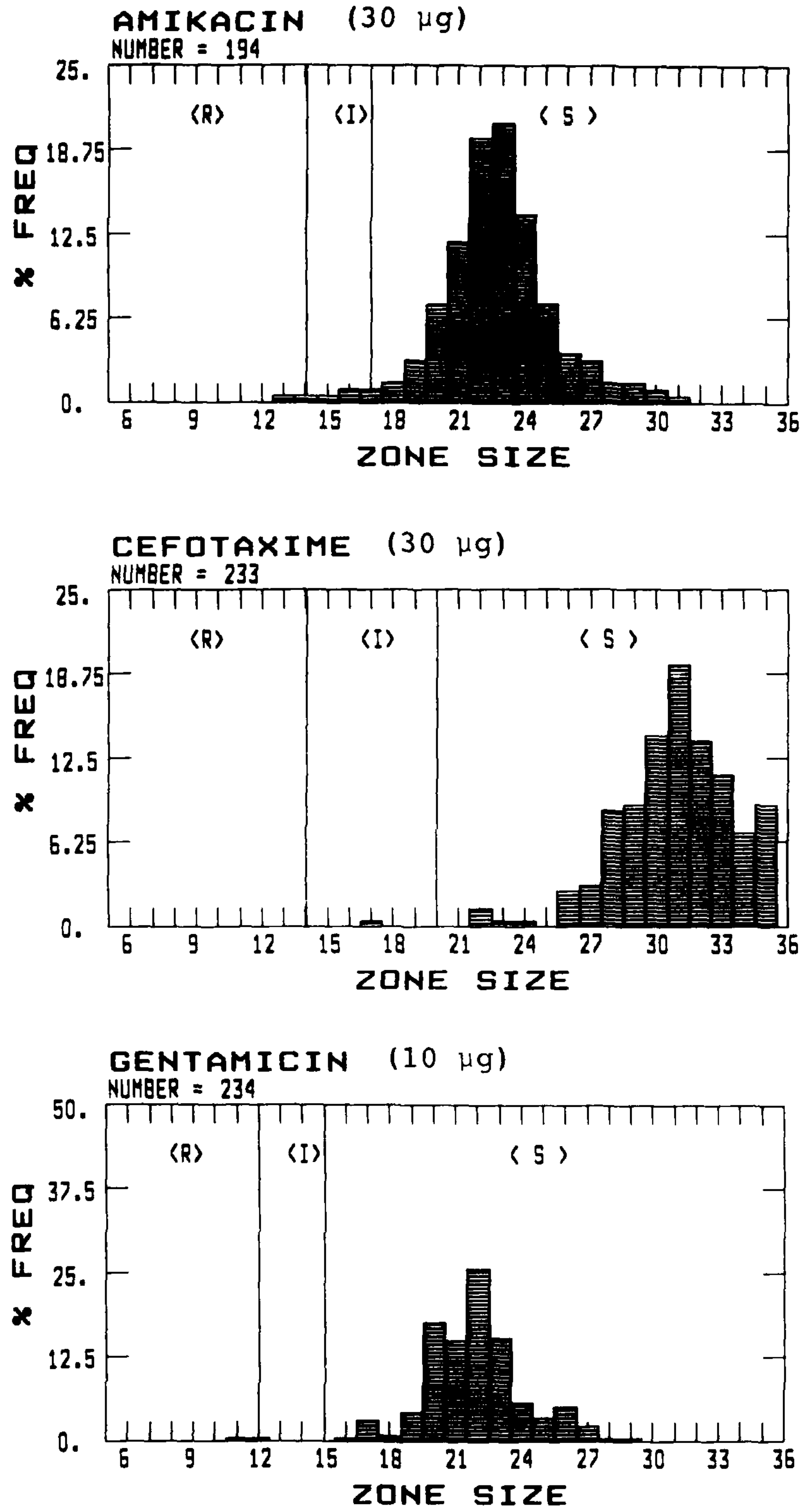
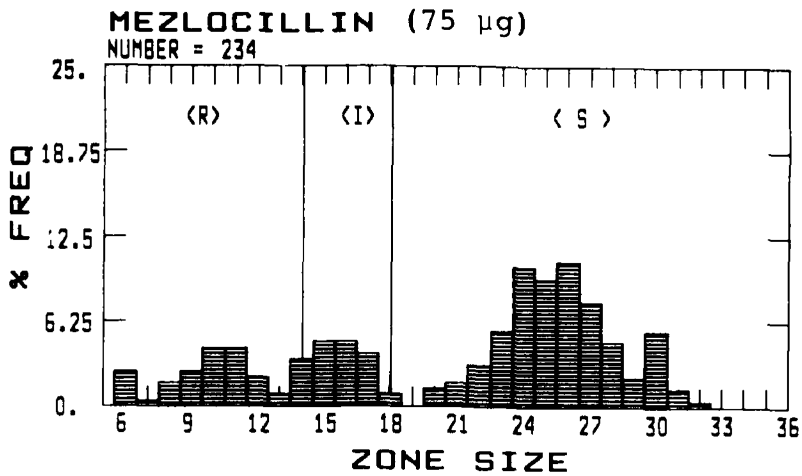
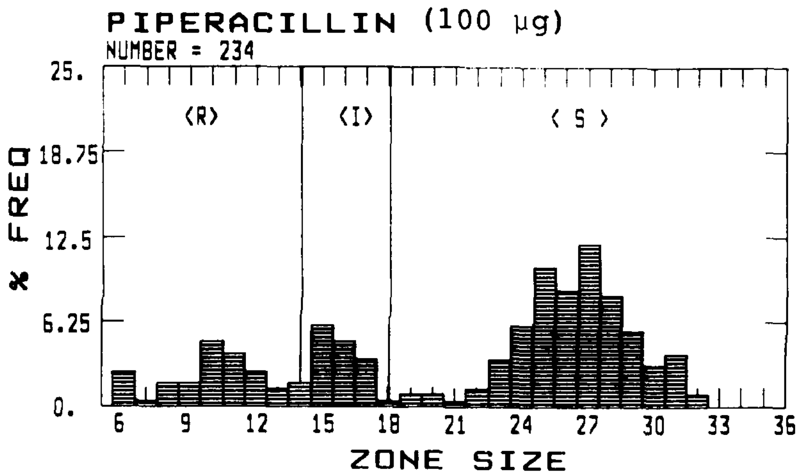


FIGURE 21. Histogram display of the distribution of zones of inhibition of growth of Escherichia coli.





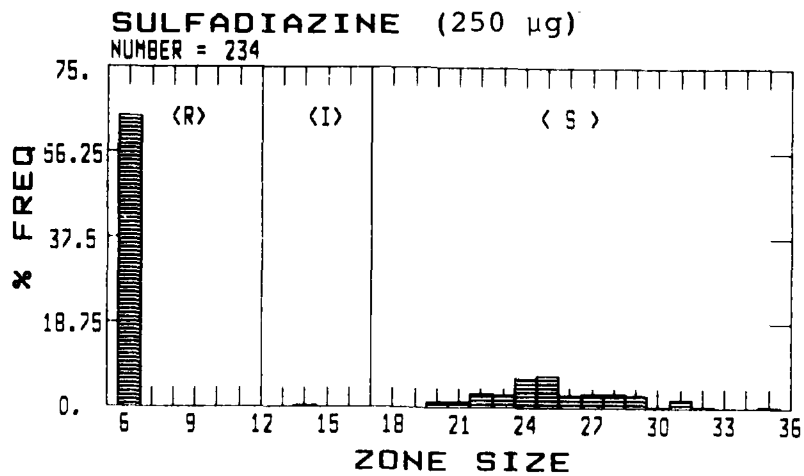


FIGURE 21. Histogram display of the distribution of zones of inhibition of growth of Escherichia coli (continued).

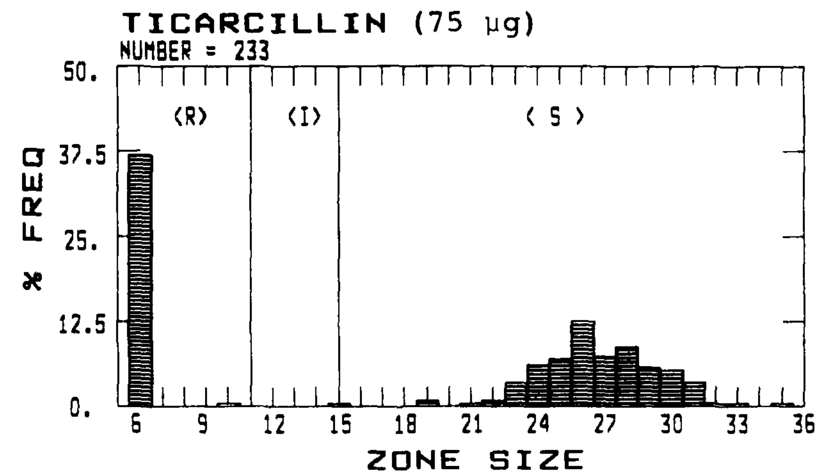


FIGURE 21. Histogram display of the distribution of zones of inhibition of growth of <u>Escherichia coli</u> (continued).

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: A Clinical Study of the Efficacy of Ceftazidime (Fortaz[R]) in the Parenteral Therapy of Infections in Hospitalized Burn Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Leslie B. Scorza, MD, Captain, MC
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: **STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY:** A Clinical Study of the Efficacy of Ceftazidime (Fortaz[R]) in the Parenteral Therapy of Infections in Hospitalized Burn Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: Leslie B. Scorza, MD, Captain, MC
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

This study is designed to evaluate the efficacy of ceftazidime in comparison to agents currently used in combination for treatment of acute bacterial infections in burned patients (piperacillin, amikacin, and/or vancomycin). Patients entered into this study are adult patients with burn injuries and clinical or laboratory indications of bacterial infection that require the use of intravenous antibiotics. The organism(s) causing the infection is proven or presumed susceptible to ceftazidime and a combination treatment prior to initiation of therapy. Patients entering this study are randomly assigned to either ceftazidime (n=25) or a drug combination (n=25). Evaluations will be made concerning the relative bacteriologic and clinical effectiveness as well as relative safety and tolerance of the treatments. The clinical and bacteriologic course of each patient is followed and documented. Laboratory and clinical data to assess safety is obtained before, during, and after treatment. Twenty-four patients have been enrolled in this study.

A CLINICAL STUDY OF THE EFFICACY OF CEFTAZIDIME (FORTAZ[R]) IN THE PARENTERAL THERAPY OF INFECTIONS IN HOSPITALIZED BURN PATIENTS

Ceftazidime is an extended spectrum cephalosporin antibiotic for parenteral administration. It offers both Gram-positive activity and activity against a wide range of Gram-negative organisms, including bacteria that are resistant to first- and second-generation cephalosporins and aminoglycosides. Ceftazidime is distinguished by its anti-Pseudomonal activity. Additionally, it demonstrates excellent stability to beta lactamases produced by many clinically important organisms.

Ceftazidime exhibits an excellent pharmacokinetic profile. The antibiotic is rapidly distributed throughout the body following parenteral administration. Ceftazidime is not metabolized; excretion is via glomerular filtration. Protein binding is low. The compound has a half-life of 1.90 h, making it possible to employ the drug on an every 8-12 h schedule.

Ceftazidime has proven to be efficacious in the treatment of nosocomial and community-acquired infections. The compound has been evaluated in worldwide clinical trials in over 5,000 patients with a variety of infection problems including lower respiratory tract, urinary tract, bone and joint, intra-abdominal, gynecologic, skin, and skin structure infections as well as bacterial septicemia and meningitis. Overall, 93% of patients treated in United States trials were clinically cured or improved (cured = 68%, improved = 25%). A bacterial cure (eradication of the initial pathogen) was achieved in 89% of ceftazidime-susceptible organisms isolated in United States clinical studies.

The objective of this study is to evaluate the efficacy of ceftazidime as monotherapy in the treatment of serious infections in burned patients and to compare the efficacy of ceftazidime to antibacterial agents currently used in combination in the treatment of serious infections in burned patients.

MATERIALS AND METHODS

Study Design. This study will randomize 50 consecutive burned patients requiring the first use of intravenous therapy for bacterial infection. Twenty-five patients will be assigned ceftazidime and 25 patients will be assigned to a combination therapy. Patients randomized to ceftazidime or to a combination that does not include vancomycin that develop a clinical need for the addition of vancomycin will be considered failures of the initial therapy. The assigned treatment will be maintained (plus vancomycin) for 8-14 days. Patients under

study who require surgery will be maintained on the assigned treatment during the perioperative period.

Selection of Patients. Patients who have clinical and/or laboratory indications of bacterial infection are eligible for entry into the study. Intravenous antibiotic therapy must be indicated. Bacteria isolated from the site of infection must be sensitive or presumed sensitive to ceftazidime and a combination therapy prior to randomization. Patients who have a history of allergy or adverse reaction to penicillins, cephalosporins, aminoglycosides, or vancomycin are excluded. Also, patients who have been treated for bacterial infection with intravenous antibiotics (except penicillin) during the current hospitalization are excluded.

Procedures Before Treatment. A chest roentgenogram and a medical history are obtained within 72 h prior to the initiation of treatment and a pertinent physical examination is performed. A laboratory profile, to include hematology, standard chemistries, and urinalysis, is obtained.

Randomization. Randomization is by a random number chart maintained in the pharmacy.

Dosage and Administration. Ceftazidime is administered in a dose of 250, 500, 750, or 1,000 mg 2-4 times daily, with the usual dose being 1,000 mg every 8-12 h. Standard therapy is administered as is currently practiced. The amikacin dose is based on 1.5 mg/kg/day divided into 2-3 equal doses per day given at equal intervals and adjusted as appropriate for renal function. The total daily dose of amikacin does not exceed 1.5 mg/kg/day. Piperacillin is administered in a dose of 3-4 g every 4-6 h not to exceed 24 g/day. Vancomycin is given as 500 mg every 6 h or 1 g every 12 h.

Procedures During Treatment. Clinical signs and symptoms of infection are assessed daily and are used as a measure of the efficacy of the study treatment. Serial chest roentgenograms and other procedures are performed as appropriate to assess clinical status. All adverse effects are recorded. Cultures are taken from the site of infection on a daily basis, with the exception of blood cultures which are taken for clinical indications.

Procedures Following Treatment. The clinical effectiveness of the assigned treatment is documented upon completion or withdrawal of treatment. This record indicates the clinical result of the original treatment and reasons, if any, for withdrawal. Seven to 10 days after completion of the treatment, a laboratory profile, to include hematology, standard chemistries, and urinalysis, and a pertinent physical examination are performed.

RESULTS

Twenty-four patients have been entered into this study. Ten of these patients expired during hospitalization at this Institute. However, all of these patients had completed a course of therapy. One patient was withdrawn and one patient was later determined ineligible for participation in the study. Of the remaining 22 patients, 10 were randomized to the combination therapy group and 12 were randomized to the ceftazidime group. The majority of patients placed on ceftazidime required the addition of vancomycin and therefore must be deemed failures of single-agent ceftazidime therapy. One patient in the combination therapy group developed worsening renal function. Otherwise, there have been no obvious side effects noted as yet.

DISCUSSION

Although it is too early at this point to draw statistically significant conclusions, there have been several patients randomized to the ceftazidime group who required the addition of vancomycin. Vancomycin was added when there was lack of improvement as documented either by microbiological or clinical means. Once vancomycin was added, improvements were noted in each patient.

PRESENTATIONS/PUBLICATIONS

None.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Evaluation of Imipenem-Cilastatin Sodium (Primaxin®) for Prophylactic Activity Against Bacterial Pneumonias in Burned Patients with Inhalation Injury: A Prospective Randomized Trial

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Leslie B. Scorza, MD, Captain, MC
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: **STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY:** Evaluation of Imipenem-Cilastatin Sodium (Primaxin[R]) for Prophylactic Activity Against Bacterial Pneumonias in Burned Patients with Inhalation Injury: A Prospective Randomized Trial

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: Leslie B. Scorza, MD, Captain, MC
Albert T. McManus, PhD
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

This study is designed to evaluate the efficacy of imipenem-cilastatin sodium for prophylactic activity against bacterial pneumonias in burned patients with inhalation injury. Patients enrolled in the study receive standard appropriate therapy for their burns and are randomized in pairs to receive or not receive prophylaxis with imipenem-cilastatin sodium. Patient pairs are then evaluated for development of pneumonia within the first 10 days (during antibiotic administration), development of pneumonia within 30 days postburn, and death or discharge from the hospital.

EVALUATION OF IMIPENEM-CILASTATIN SODIUM (PRIMAXIN^{R}) FOR PROPHYLACTIC ACTIVITY AGAINST BACTERIAL PNEUMONIAS IN BURNED PATIENTS WITH INHALATION INJURY: A PROSPECTIVE RANDOMIZED TRIAL

Inhalation injury is an important problem in the care of the burn patient. It increases the mortality of burn injury and causes the largest effect in burns of moderate size. In the most recent review, inhalation injury was found in 35% of patients and bacterial pneumonia in 19% (1). Inhalation injury also seems to predispose to the development of bacterial pneumonia, with 45.8% of patients with severe inhalation injury developing pneumonia. In this analysis, patients with inhalation injury who developed pneumonia more commonly showed the pneumonia in the first week after injury.

Unfortunately, there is no specifically effective treatment of inhalation injury. Patients are supported according to their clinical status with appropriate fluid administration, maintenance of airway patency, adequate amounts of oxygen, and mechanical ventilation as necessary. Some patients tolerate the insult well and recover rapidly; however, others develop pneumonia, initially with *Staphylococcus aureus* and later with Gram-negative organisms, which can lead to progressive respiratory failure and death.

Prophylactic treatment against the sequelae of inhalation injury may be useful as there is no other therapy available to prevent bacterial pneumonia. Levine *et al* (2) evaluated the effect of prophylactic aerosolized gentamicin in patients with inhalation injury and found no difference in mortality, time of death, or pulmonary or septic complications. Gram-negative pneumonias were the most common presentation in their work; however, more recently, Gram-positive pneumonia usually precedes the development of Gram-negative pneumonia.

Imipenem-cilastatin sodium (Primaxin^{R}), a thienamycin antibiotic which has been recently released, could be of use in the prevention of this problem. This antibiotic has a wide spectrum, being active against Gram-positive, Gram-negative, and anaerobic bacteria. It is bactericidal even against aminoglycoside resistant and beta-lactamase producing organisms. It is effective against strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Only strains of *Pseudomonas maltophilia*, *Pseudomonas cepacia*, *Streptococcus faecium*, flavobacteria, and diptheroids have been found to be resistant to imipenem-cilastatin sodium. Minimal toxicity has been attributed to the drug, which is excreted by the kidneys; dosage reductions to one-third are required in anuric patients. Therefore, it is a good choice for prophylactic therapy for inhalation injury.

Prophylactic treatment that has been previously tried was principally directed toward wound infection and offered antibiotics that were more inherently toxic without the wide spectrum of imipenem-cilastatin sodium. Antibiotics have also been administered into the tracheobronchial tree without beneficial effects. The regimen proposed offers the advantage of an intravenous agent with good penetration of lung tissue, minimal toxicity, and a wide spectrum against the organisms most often involved in the pneumonias associated with inhalation injury. Randomization will be employed to compare this new prophylactic regimen with the usual expectant treatment.

The purpose of this study is to evaluate prophylactic treatment of inhalation injury and correlate with the clinical prevention of pneumonia in such patients.

MATERIALS AND METHODS

Number of Patients. Up to 200 patients will be entered into this study, with an early cutoff by closed-end sequential analysis possible.

Criteria for Admission to the Study. Patients admitted to the US Army Institute of Surgical Research with evidence of inhalation injury will be offered the opportunity to participate in a study of prophylactic administration of imipenem-cilastatin sodium. Patients having the following will be considered for enrollment in the study:

1. A history of inhalation of smoke and/or flames.

2. A history of burn occurring in a closed space with the physical findings of burns to the face, lips, nose, or mouth or singeing of facial or nasal hair.

3. Carbonaceous sputum production.

4. Stridor, hoarseness, or airway obstruction.

5. Dyspnea, wheezing, or rhonchi.

6. Xenon scan showing trapping of gas in a pattern consistent with inhalation injury.

Patient Inclusion. Male and female patients will be selected for participation in the study if they meet the following criteria:

1. The patient is at least 18 yr old.

2. The diagnosis of inhalation injury is confirmed by bronchoscopic examination.

3. The patient has an expected probability of survival of 20-80% based on an age and burn size predictor model.

4. The patient is not subject to any preexisting disease which would contraindicate administration of imipenem-cilastatin sodium.

5. The patient or representative signs the appropriate informed consent.

6. The patient's treatment can begin by 72 h postburn.

Patient Exclusion. Patients with the following characteristics will be excluded from participation in the study:

1. Patients < 18 yr old.

2. Patients who are pregnant or nursing.

3. Patients who have a prior history of renal dysfunction.

4. Patients who are hypersensitive to thienamycin or who have had an anaphylactic reaction to any of the beta-lactam groups of antibiotics, including cephalosporins, oxacephalosporins, penicillins, or cephamycins.

5. Patients with prosthetic valve endocarditis.

6. Patients in danger of or in a hepatic coma.

Study Design. Patients will receive standard appropriate therapy for their burn and will be randomized in pairs to receive or not receive prophylaxis with imipenem-cilastatin sodium. Imipenem-cilastatin sodium at 500 mg every 6 h will be started between 36 and 72 h postburn and administered for 240 h (10 days). No other modifications will be made in the care of either group of patients. Sputum cultures and other cultures will be collected according to the usual protocol for all patients. Respiratory status will be carefully monitored according to the usual procedures in all patients and documented appropriately.

Patients in both groups will be carefully watched for the development of pulmonary infection. Tracheobronchitis and pneumonia will be diagnosed by the usual criteria (Table 1) and will be treated with antibiotics when judged appropriate by the primary physician. Cellulitis diagnosed by clinical criteria and treated with penicillin will not be grounds for exclusion from the study. Patient pairs will be excluded from the study if either patient develops a deep tissue infection requiring treatment with antibiotics or succumbs without pneumonia in the

TABLE 1. Diagnosis of Infection

Pneumonia
1. Clinical findings consistent with pneumonia, i.e., pleuritic chest pain, fever, purulent sputum, or other signs of sepsis.
2. A significant number (> 25) of PMLs on methylene blue stain of endotracheal secretions with < 25 squamous epithelial cells per 100X field.
3. Roentgenographic findings consistent with pneumonia.
4. Positive sputum culture (confirmatory, but not essential for diagnosis).

Tracheobronchitis
1. Clinical findings consistent with the diagnosis, i.e., fever, purulent sputum, sepsis, or significant findings on bronchoscopy.
2. A significant number (> 25) of PMLs on methylene blue stain of endotracheal secretions with < 25 squamous epithelial cells per 100X field.
3. Roentgenographic findings <u>not</u> consistent with pneumonia.
4. Positive sputum culture (confirmatory, but not essential for diagnosis).

first 10 days. Patient pairs will be evaluated for the development of pneumonia within the first 10 days (during antibiotic administration), pneumonia developing within 30 days postburn, and death or discharge from hospital.

Patients will be entered into the study as sequential pairs with treatment randomly allocated between the paired patients. This will allow sequential analysis with a closed-end statistical model (3). Based on the probability of the untreated group experiencing pneumonia (45% from past clinical experience), a significance level of 0.05, a power of 0.75, and an improvement with therapy to half the untreated value, the maximum number of patients theoretically required can be estimated to be 82 patients per group (Fig 1). If imipenem-cilastatin sodium therapy proves to be either very

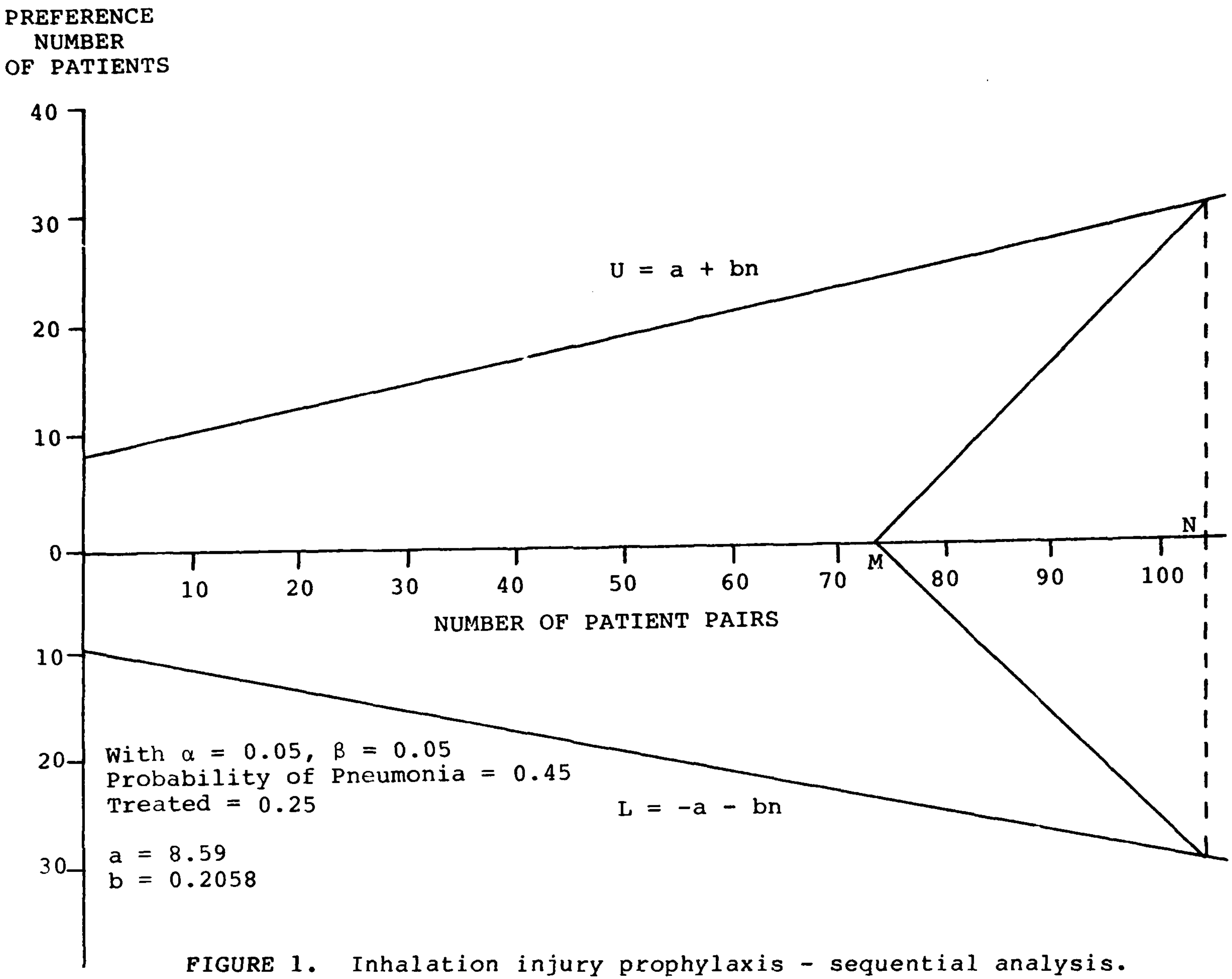


FIGURE 1. Inhalation injury prophylaxis - sequential analysis.

effective or detrimental, the sequential analysis will allow the study to be completed with significantly fewer patients.

RESULTS

Because the population for this study conflicts with another study, no patients have been entered into this study to date.

DISCUSSION

When 50 patients have completed the study, the data will be analyzed as to the prophylactic activity of imipenem-cilastatin sodium against bacterial pneumonias.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. Shirani KZ, Pruitt BA Jr, and Mason AD Jr: The influence of inhalation injury and pneumonia on burn mortality. **Ann Surg** 205:82-7, 1987.

2. Levine BA, Petroff PA, Slade CL, et al: Prospective trials of dexamethasone and aerosolized gentamicin in the treatment of inhalation injury in the burned patient. **J Trauma** 18:188-93, 1978.

3. Armitage P: **Sequential Medical Trials.** Springfield: CC Thomas Publishers, 1960.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA314656	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
88 05 13	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	F	302
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Investigation of the Physiologic and Immunologic Effects of Prostaglandin E in Septic and Traumatized Rats

12. SUBJECT AREAS
06 05 Medicine and Medical Research 06 16 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
88 05	90 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Basil A. Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.3	15
89	0.5	30

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR WAYMACK, J P
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-3411
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) MASON, A D
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) MC MANUS, A T

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Prostaglandin; (U) Physiological Effects; (U) Immunosuppression; (U) Pharmacology; (U) Trauma; (U) Septicemia; (U) Lab

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) Animals: (U) Rats; (U) RAII

23. (U) To determine the physiologic and immunologic effects of the elevations in prostaglandin E levels seen following trauma, sepsis, and tumor growth, to determine whether the net effect of the elevation in prostaglandin E levels of these disease states is beneficial or detrimental, and to determine whether pharmacologic manipulation of the prostaglandin E level can be beneficial in terms of immunologic or physiologic function. A literature search was performed and indicated no duplication of effort.

24. (U) Healthy adult Lewis rats will be administered varying doses of the long-acting prostaglandin E derivative, 16,16-dimethyl-prostaglandin E, prior to receiving a number of physiologic challenges. Initial evaluations will center on the ability of prostaglandin E to alter resistance to mortality following challenge with endotoxin.

25. (U) 8805 - 8809. This project was approved by the USAISR Research Council and the US Army Institute of Surgical Research Animal Care and Use Committee in April 1988. A lethal endotoxin model was developed and investigations have begun on the effect of prostaglandin E on resistance to endotoxin shock.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Investigation of the Physiologic and Immunologic Function of Prostaglandin E in Septic and Traumatized Rats

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

J. Paul Waymack, MD, Major, MC
Rey F. Guzman, SGT
Carlin V. Okerberg, DVM, PhD, Major, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

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Basil A. Pruitt, Jr., MD, Colonel, MC

Administration of a long-acting prostaglandin E, 16,16-dimethyl-prostaglandin E (dPGE), to rats improved their survival of bacterial peritonitis. We examined the mechanism of this protective effect with reference to its interaction with the release of cachectin (TNF). Sixty rats received either saline, 20 $\mu g/kg$ dPGE, or 80 $\mu g/kg$ dPGE 12 h prior to endotoxin and continuing for 48 h. Survival rates for the saline, 20 $\mu g/kg$ dPGE, and 80 $\mu g/kg$ dPGE groups were 0%, 40%, and 85%, respectively. Forty rats received saline or 80 $\mu g/kg$ dPGE, with the initial dose being 3 h following endotoxin challenge and continuing for 48 h. Survival rates for both groups were 0%. Sixty rats received saline or 80 $\mu g/kg$ dPGE at 12 h and 1 h prior to endotoxin challenge. Two h later, they were sacrificed and plasma TNF levels were assayed. Plasma TNF levels for the control group was 22.72 ± 0.83 ng/ml and for the treatment group, 16.03 ± 1.13 ng/ml ($P < 0.001$). It appears that one of prostaglandin E's physiologic roles is to decrease TNF production during sepsis and thereby protect against endotoxin shock.

There is conflicting data on the effect of indomethacin on resistance to sepsis, possibly due to its ability to affect multiple components of the immune system, including physiologic interactions. We examined the effect of indomethacin on resistance to endotoxin shock with special reference to the rate of TNF appearance. Seventy-five rats received either 1 ml saline or 4 mg/kg indomethacin every 12 h commencing 12 h prior to endotoxin challenge and continuing for 2 days. The mortality rates were 15% for the control group and 46% for the treatment group ($P < 0.01$). The mean survival times were 147.7 ± 7.8 and 102.9 ± 12.2 h, respectively ($P < 0.001$). Sixty additional animals received either saline or indomethacin

at 12 h and 1 h prior to endotoxin challenge. Two hours later, plasma was obtained for analysis of TNF levels. TNF levels for control and treatment groups were 22.72 $\pm$ 0.83 and 21.97 $\pm$ 1.28 ng/ml ($P = 0.61$). Indomethacin appears to decrease resistance to endotoxin shock, but not through alterations in the rate of TNF appearance. Administration of indomethacin to septic patients in an attempt to improve immune function may result in endotoxin deaths.

INVESTIGATION OF THE PHYSIOLOGIC AND IMMUNOLOGIC FUNCTION OF PROSTAGLANDIN E IN SEPTIC AND TRAUMATIZED RATS

Infection is a major cause of morbidity and mortality in trauma patients who survive the initial 24 h postinjury. This infection diathesis is related to disruption of normal epithelial barriers by penetrating wounds or burns and the posttraumatic immunosuppression (1).

Postinjury immunosuppression involves nearly the entire immune system (2,3). Its exact etiology has not been completely elucidated; however, a most frequently espoused explanation is that the injury results in the release of potent immunosuppressive metabolites (4). The most extensively investigated of these metabolites is prostaglandin E (PGE) (5).

PGE has been demonstrated to impair immune function in a number of *in vitro* leukocyte culture models (6). These models involved adding PGE to WBC cultures and assaying various leukocyte functions. An immunosuppressive effect of PGE is also apparent when PGE synthesis is blocked in traumatized animals by the administration of cyclooxygenase inhibitors (7,8). These studies indicated that the administration of cyclooxygenase inhibitors corrected the posttraumatic immunosuppression and increased survival rates following a septic challenge.

Conversely, it has more recently been reported that the parenteral administration of a long-activing PGE derivative, 16,16-dimethyl-prostaglandin E (dPGE), enhanced survival rates in rats subjected to an *Escherichia coli* peritonitis (9). In an attempt to determine the mechanism of the protective effects of dPGE, the effect of parenterally administered dPGE on resistance to endotoxin shock was assayed. The effect of pharmacologic blockade of PGE synthesis on resistance to endotoxin shock was also assayed.

MATERIALS AND METHODS

Animals. Three hundred and sixty-five adult male Lewis rats weighing ± 250 g were used for these studies. The animals were housed in individual stainless steel, hanging cages and allowed food and water *ad libitum*. The animals were observed for a minimum of one week prior to entry into the study to exclude the presence of any preexisting diseases.

Endotoxin Model. The endotoxin model was produced by the intravenous injection of either 1 X 10^9 or 1 X 10^{10} heat-killed *Escherichia coli* organisms. The *Escherichia coli* were cultured in trypticase soy broth at 37°C for 16 h. The culture was then placed in a 95°C water bath for 1 h immediately prior to injection. In previous studies, *Escherichia coli* prepared in

this fashion caused a 100% kill rate. The heat-killed bacteria were centrifuged and resuspended in sufficient normal saline to achieve a final concentration of either 1 X 10^9 or 1 X 10^{10} Escherichia coli per milliliter saline. The suspension was injected through a 25-ga needle into the dorsal penile vein.

Drugs. The dPGE was generously supplied by The Upjohn Company (Kalamazoo MI) and was injected intraperitoneally through a 25-ga needle. It was diluted with sufficient normal saline to achieve final concentrations of dPGE that permitted the desired dose of the drug to be administered in a final volume of 1 ml.

Indomethacin sodium trihydrate was generously supplied by Merck Sharp & Dohme Research Laboratories (Somerset NJ). The indomethacin was dissolved in sufficient normal saline to achieve a final concentration of 1 mg/ml and administered intraperitoneally at a dosage of 4 mg/kg.

Mortality Studies. For the first phase of the study, 60 rats were randomized to three groups. The first group (n=20) received 1 ml of normal saline by intraperitoneal injection every 12 h commencing 12 h prior to endotoxin challenge and continuing for a period of 48 h. The second group (n=20) received 20 µg/kg dPGE and the final group (n=20), 80 µg/kg dPGE on the same dosage schedule. Twelve hours after the initial injection of either saline or dPGE, the animals were injected with 1 X 10^{10} heat-killed Escherichia coli.

For the second phase of the study, 40 rats were randomized to either a saline control group or an 80 µg/kg dPGE treatment group. The animals were injected intravenously with 1 X 10^{10} heat-killed Escherichia coli organisms and received their initial treatment 3 h following endotoxin challenge. These animals received either the saline or dPGE every 12 h for a total of 4 doses.

For the third phase of the study, 75 rats were divided into a saline control group (n=40) and an indomethacin treatment group (n=35). The control group received twice daily injections of 1 ml normal saline commencing 12 h prior to endotoxin challenge and continuing for 2 days. The treatment group received twice daily injections of 4 mg/kg indomethacin commencing 12 h prior to endotoxin challenge and continuing for 2 days. Twelve hours after the initial injection of either saline or indomethacin, the animals were injected with 1 X 10^9 heat-killed Escherichia coli organisms.

All animals were followed for 7 days after challenge to determine mean survival times and absolute survival rates. For the calculation of mean survival times, animals surviving 7 days were given a survival time of 168 h. Animals were also observed for the development of diarrhea during the period from

the initial administration of the drug until endotoxin challenge. Diarrhea was defined as the presence of loose, nonformed bowel movements.

Histopathology Studies. In the fourth phase of this study, 20 rats were randomized to receive either saline (n=10) or 80 μg/kg dPGE (n=10) commencing 12 h prior to administration of 1 X 10^{10} heat-killed Escherichia coli organisms. These animals received a second dose of saline or dPGE immediately prior to endotoxin challenge and were sacrificed 6 h after challenge by decapitation.

Twenty additional rats were randomized to a saline control group (n=10) and an indomethacin treatment group (n=10). The control group received 1 ml of normal saline intraperitoneally 12 h and immediately prior to endotoxin challenge. The treatment group received 4 mg/kg indomethacin at the same time periods. The endotoxin was again administered in the form of 1 X 10^{10} heat-killed Escherichia coli organisms suspended in 1 ml normal saline injected through the dorsal penile vein. An additional group (n=10) received the same dosage of indomethacin using the same time sequence but was not challenged with endotoxin. Six hours after endotoxin challenge, the animals were sacrificed.

The lungs of these animals were rapidly excised, weighed, and placed in formalin for permanent histologic examination. The kidneys, stomach, and small intestine were also removed, grossly inspected, and placed in formalin. All tissues subsequently underwent standard permanent histologic processing using H&E stain. These histologic specimens were examined for evidence of infarction, hemorrhage, edema, and ulceration.

Tumor Necrosis Factor (TNF) Assays. For the last phase of this study, 60 rats were administered either saline (n=40) or 80 μg/kg dPGE (n=20) 12 h and 1 h prior to challenge with 1 X 10^{10} heat-killed Escherichia coli organisms. Another 60 rats were randomized to a saline control group (n=40) and an indomethacin treatment group (n=20). The control group received 1 ml of normal saline intraperitoneally 12 h and immediately prior to challenge with 1 X 10^{10} heat-killed Escherichia coli organisms. The treatment group received 4 mg/kg indomethacin 12 h and immediately prior to being challenged with 1 X 10^{10} heat-killed Escherichia coli organisms. Two hours after challenge, the animals were sacrificed by decapitation and blood samples obtained. The blood was placed in heparinized tubes and centrifuged at 2000 rpm for 10 min. The plasma was collected and stored at -70°C until assayed for TNF levels using a murine L-929 fibroblast cytotoxicity assay as previously described (10). The detection limit of this assay was 500 ng/l plasma.

Statistical Analyses. All data are presented as mean ± SEM. Comparison between groups was performed using ANOVA, student's t test, Chi square, Kruskal-Wallis test, or the Wilcoxon test, as appropriate.

RESULTS

In the animals treated with dPGE or saline prior to the intravenous heat-killed _Escherichia coli_ challenge, the mean survival time for the saline-treated group was 21.4 ± 0.7 h (Fig 1). Those animals that received 20 μg/kg dPGE had a mean survival time of 81.4 ± 16.3 h, and those that received 80 μg/kg dPGE had a mean survival time of 148.9 ± 10.7 h. These differences were statistically significant ($P < 0.0001$, Kruskal-Wallis test). The absolute survival rates for these animals were 0% for the saline-treated group, 40% for the 20 μg/kg dPGE-treated group, and 85% for the 80 μg/kg dPGE-treated group. These differences were also statistically significant ($P < 0.001$, Chi square).

Among the 20 rats administered saline intraperitoneally prior to endotoxin challenge, 5 (25%) developed diarrhea before the administration of the endotoxin. Diarrhea developed in 8 of the 20 animals (40%) administered 20 μg/kg dPGE, and in 20 of 20 animals (100%) given 80 μg/kg dPGE. This increased incidence of diarrhea was statistically significant ($P < 0.005$, Chi square).

There was no effect on mortality noted when dPGE was administered after endotoxin challenge. The mean survival time of the animals receiving saline commencing 3 h following endotoxin challenge was 20.6 ± 2.4 h (Fig 2). For the animals given their initial dose of 80 μg/kgdPGE 3 h following endotoxin challenge, the mean survival time was 20.1 ± 1.1 h. This difference was not statistically significant ($P = 0.83$, Wilcoxon test). Both of these groups had a 0% survival rate.

In the indomethacin mortality group, the control group had a 15% mortality rate. Those animals that received indomethacin therapy had a 46% mortality rate (Fig 3). These differences were statistically significant ($P < 0.01$, Chi square). The mean survival time for the control group was 147.7 ± 7.8 h and for the treatment group, 102.9 ± 12.2 h. These differences were also statistically significant ($P < 0.001$, Wilcoxon test).

The mean lung weight of saline-treated animals was 1.40 ± 0.05 g and for the lungs obtained from dPGE-treated animals, 1.35 ± 0.04 g ($P = 0.413$, student's t test). Six of the 10 pulmonary specimens from both saline-treated and dPGE-treated animals showed histologic evidence of pulmonary hemorrhage and edema. There was no gross or histologic evidence of intestinal infarction or ulceration in either of

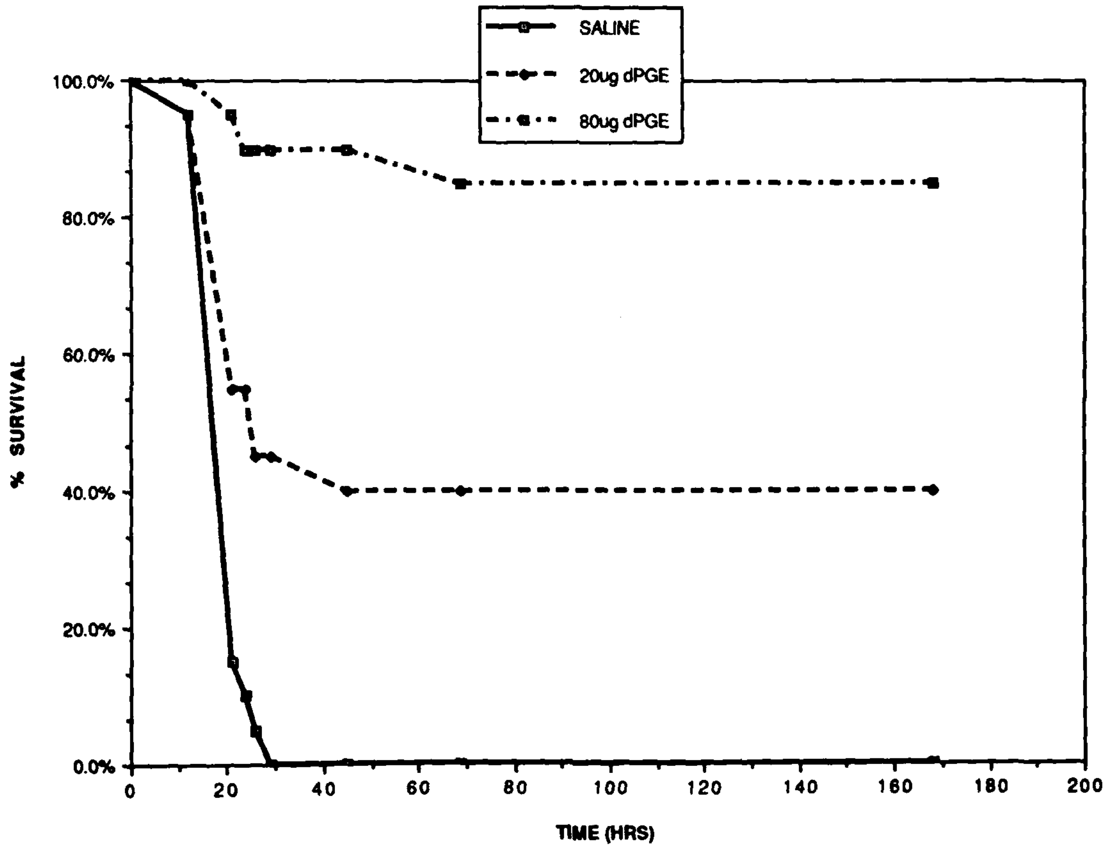

FIGURE 1. Survival curves for animals treated with saline, 20 μg/kg dPGE, or 80 μg/kg dPGE prior to endotoxin challenge.

the groups. There also was no evidence of gastric ulceration or renal hemorrhage and necrosis noted in either of the groups.

The plasma TNF level for saline-treated animals was 22.72 ± 0.83 ng/mg, for dPGE-treated animals, 16.03 ± 1.13 ng/ml, and for indomethacin-treated animals, 21.97 ± 1.28 ng/ml ($P < 0.001$, ANOVA).

DISCUSSION

Initially published papers on the effect of PGE in trauma patients suggested that PGE levels increased following traumatic injuries and that PGE was immunosuppressive and predisposed the patient to potentially lethal infections. The immunologic data which demonstrated an immunosuppressive effect of PGE were primarily of an *in vitro* nature in which PGE was added to leukocyte cell cultures and the leukocytes then assayed for various WBC functions (6). These assays demonstrated that the addition of PGE to the cultures resulted

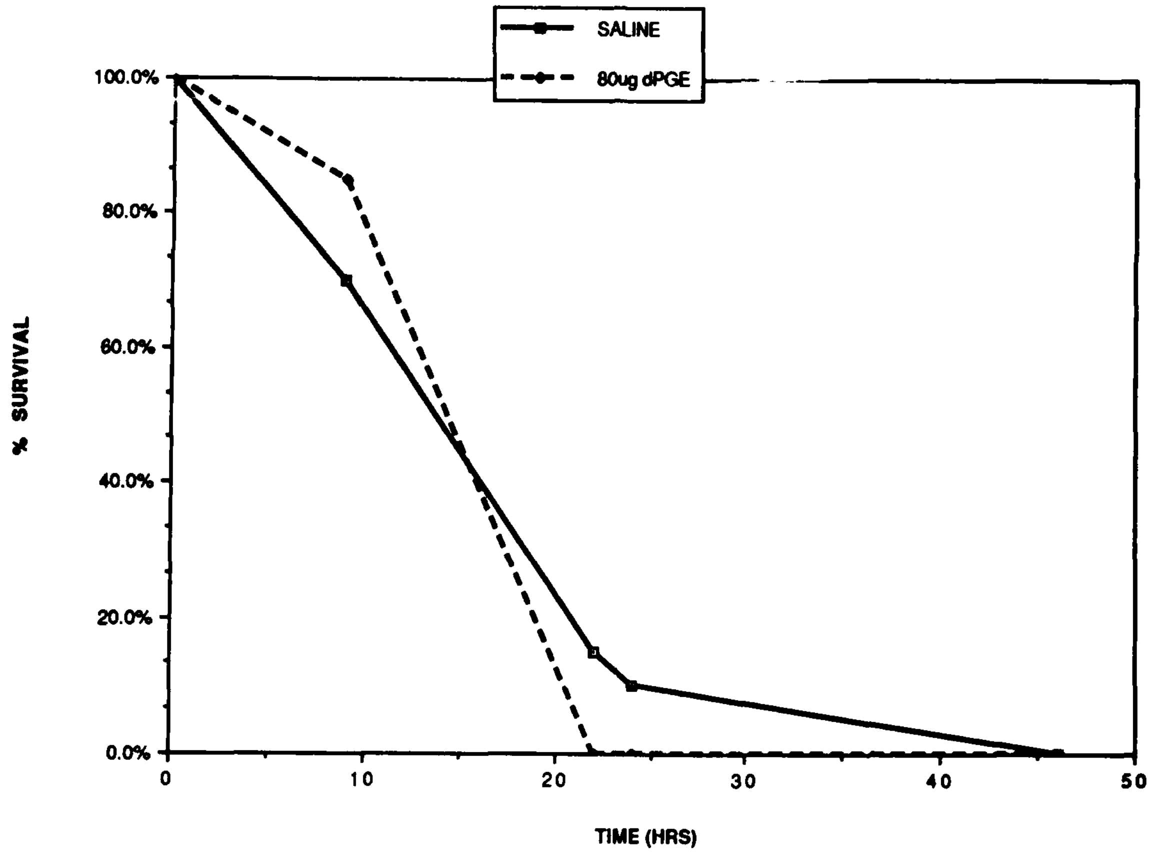

FIGURE 2. Survival curves for animals treated with saline or 80 μg/kg dPGE 3 h after endotoxin challenge.

in an impairment of lymphocyte blastogenesis, macrophage chemotaxis and phagocytosis, and many impairments in neutrophil function. There was, however, very little in vivo data available due to the extremely short half-life of PGE following parenteral administration. However, in an in vivo situation, PGE might not be detrimental to traumatized individuals and might even have beneficial effects in trauma patients at risk for developing sepsis. Such a discrepancy between in vivo and in vitro effects has previously been demonstrated to exist with such immunomodulatory agents as TP-5, which has been shown to be immunosuppressive in vitro but immunostimulatory in vivo (11-13).

Recently, a long-active derivative of PGE, 16,16-dimethyl-prostaglandin E (dPGE), has become available (14). This compound exhibits the physiologic and immunologic effects of PGE but has a far longer half-life due to the two methyl groups present on one of its hydrocarbon chains which prevent degradation by the normal enzymatic system. It

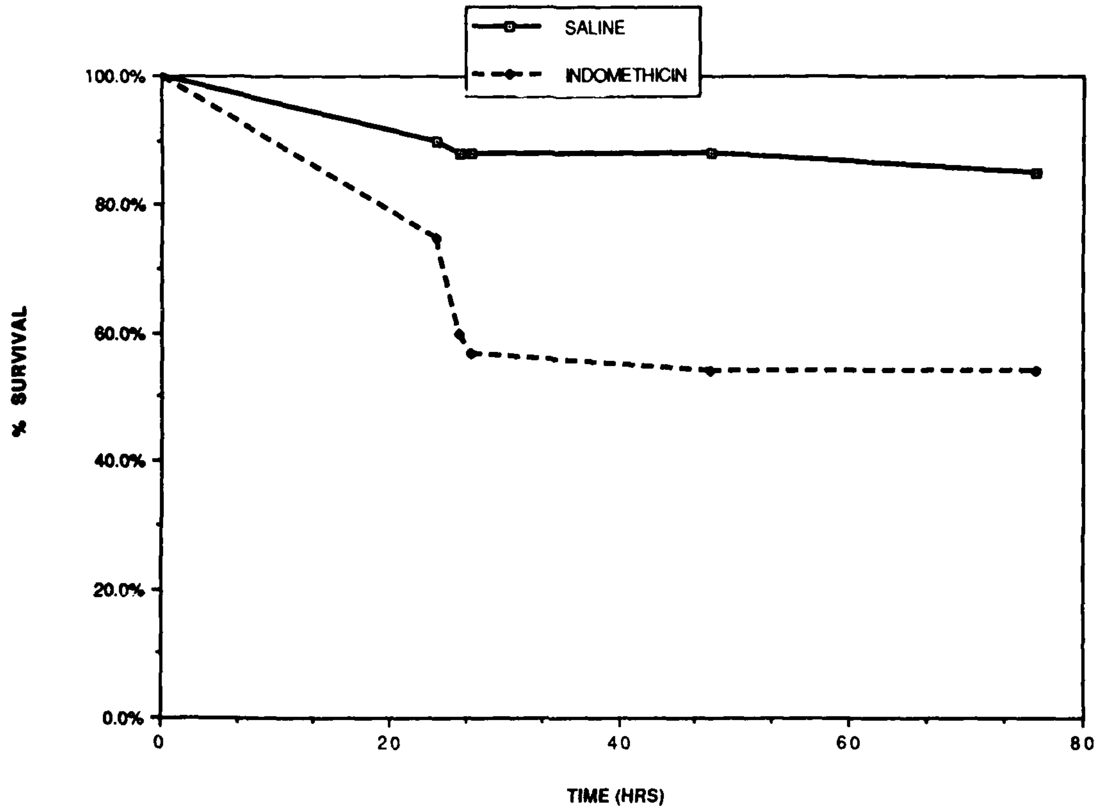

FIGURE 3. Survival curves for saline- and indomethacin-treated animals challenged with endotoxin.

therefore allows for investigation of the effect of PGE in *in vivo* models.

dPGE has recently been reported to improve survival rates in septic models when administered parenterally (9). Lewis rats administered 80 µg/kg dPGE prior to an intraperitoneal *Escherichia coli* peritonitis challenge had an increase in their mean survival time from 4.25±0.78 days to 6.80±0.45 days ($P < 0.03$). This finding suggests the possibility that PGE does indeed have some beneficial effects in the traumatized individual at risk for sepsis and that the previously cited *in vitro* data does not completely describe the *in vivo* effects of PGE.

One of the methods by which Gram-negative bacteremia results in patient death is through the endotoxin generated by such Gram-negative bacteria (15,16). Endotoxin has long been recognized to be toxic to patients and to result in multisystem organ failure. More recently, endotoxin stimulation of TNF (cachectin) secretion by host macrophages has been proposed as one of the mechanisms by which endotoxin triggers multisystem organ failure (17). Sublethal administration of TNF has been

shown to result in significant tumor regression in animal models and enhanced resistance to bacterial infections (18,19). TNF administration alters thermal regulation and produces multiphasic febrile responses as well as an elevation of plasma hematocrit levels due to a loss of intravascular fluid (17,20). TNF affects metabolism in a manner similar to sepsis, as reflected by simultaneous decrease in plasma glucose levels and increase in plasma lactate levels and stimulation of hepatic acute-phase protein synthesis (17,21). Chronic administration of TNF is associated with cachexia and muscle wasting (22). At low doses, TNF also exerts predominantly stimulative effects on WBCs (23). At higher concentrations, it can specifically precipitate fatal hemodynamic instability (24).

Our current study demonstrates a physiologically beneficial role of the elevated PGE levels seen in traumatized patients with respect to endotoxin and TNF metabolism. Our data indicate that at least part of the improved survival seen in dPGE-treated animals may be attributable to altered release of toxic metabolites, including TNF, following endotoxin challenge. This is supported by the increase in survival of endotoxin shock of from 0% to 85% if dPGE was given prior to challenge and is further corroborated by the finding that if the dPGE was not administered until 3 h following endotoxin challenge, the survival rate was the same as in the saline control group (0% vs. 0%). Since TNF is known to be produced during the initial 2 h following endotoxin challenge (25), the demonstration of a beneficial effect of dPGE when administered prior to endotoxin challenge but not 3 h following endotoxin challenge is entirely consistent with inhibition of toxic metabolite synthesis or release.

Although a significantly decreased rate of TNF appearance in animals pretreated with dPGE is evident, we suspect that the dPGE exerts protective effects in other areas of host response to endotoxin. The 29% decrease in the rate of TNF appearance in dPGE-treated animals should not be sufficient to completely account for the increase in survival rates from 0% for the control group to 85% for dPGE-treated animals. There would appear to be two additional areas in which dPGE might exert beneficial effects in endotoxin-challenged aniamls. First, dPGE might prevent the synthesis of toxic metabolites other than TNF. Such a possibility is supported by the fact that the dPGE was protective when administered prior to endotoxin challenge but not if the initial dose was given 3 h following endotoxin challenge. Alternatively, dPGE may protect at the end-organ target of such mediators. Further studies utilizing recombinant TNF plus dPGE will be necessary to answer this question definitively.

We believe that the data presented herein are consistent with a negative feedback loop for macrophage metabolism following endotoxin challenge. It has previously been

demonstrated that macrophages exposed to endotoxin synthesize TNF (17). It has been further documented that macrophages exposed to TNF synthesize PGE (26). Our finding that PGE may inhibit some components of TNF production suggests a negative feedback loop. Thus, macrophages exposed to endotoxin begin producing TNF to help fight the bacteria generating the endotoxin. In order to prevent serum levels of TNF from reaching concentrations which are harmful to the host, the TNF triggers synthesis of PGE, which may then modulate TNF production.

One area of concern is highlighted by our data. There have been a number of suggestions that traumatized and septic patients, who are recognized to be immunosuppressed, should be administered cyclooxygenase inhibitors in an effort to decrease PGE production. Although such proposals do have scientific support based on the PGE studies of Faist and others (27,28), we believe it should be recognized that inhibition of PGE synthesis may render the traumatized patient at increased risk for death from elevated TNF levels due to inhibition of the negative feedback loop of macrophage TNF-PGE metabolism. We therefore recommend close observation of septic patients given cyclooxygenase inhibitors in order to identify complications due to elevated TNF levels. Further studies will obviously be needed to determine whether the potential immunostimulatory role of cyclooxygenase inhibitors or the potential endotoxin protective effect of PGE is more critical in traumatized patients.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG6968	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	F	303
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code)
(U) Alteration of Host Resistance in Burned Soldiers

12. SUBJECT AREAS
06 05 Medicine and Medical Research 06 13 Microbiology 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
76 10	99 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	5.0	250
89	5.0	263

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, A T
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-3411
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Tissue-Spreading Factors; (U) Infection; (U) Immunostimulants; (U) Virulence Factors; (U) Plasmids; (U) Antibiotic

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) Effects; (U) Volunteers: (U)Adults; (U) Children; (U) RAII

23. (U) To define the microbial basis of opportunistic infection in susceptible burned soldiers, identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens, and develop and evaluate countermeasures. A literature search was performed and indicated no duplication of effort.

24. (U) The high susceptibility of burned rats to experimental infection with Pseudomonas aeruginosa and Proteus mirabilis will be investigated. The effect of in vitro alterations of specific microbial characteristics on infection will be investigated. Specific antimicrobial and immunostimulatory therapies will be examined.

25. (U) 8710 - 8712. The clinical trial of the parenteral antibiotic ceftazidime as monotherapy in infected burn patients is continuing. A newly described beta-lactamase with activity against most of the third-generation cephalosporins has been identified in several isolates. The gene coding for the enzyme appears chromosomal. The enzyme has an isoelectric point of 6.3. Investigations into the mechanisms of antimicrobial activity of mafenide acetate have shown the compound not to be an antagonist of dihydropteroate synthetase. This finding confirms other biologic data that mafenide acetate is not a typical sulfonamide.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Alteration of Host Resistance in Burned Soldiers

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Albert T. McManus, PhD
Virginia C. English, MS
Camille L. Denton, MA
Charles H. Guymon, MS, SGT
Aldo H. Reyes, SSG
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

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Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

A clinical trial and *in vitro* evaluation of the parenteral antibiotic ceftazidime as monotherapy in infected burn patients is in progress. Twelve patients have been entered into the study. Sensitivity of Gram-negative organisms was 85%, which is a significant drop from the previous reporting period (93%, $P < 0.01$). This resistance change is largely due to a new beta-lactamase with a wide activity among third-generation cephalosporins. An enzyme of chromosomal origin with an isoelectric point of 6.3 has been found in resistant strains. A candidate immunopotentiating agent, sodium hyaluronate, was tested and found not be active against experimental Pseudomonas burn wound sepsis. Serotyping of *Pseudomonas aeruginosa* isolates showed no evidence of the accumulation of endemic organisms.

ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

Experimental Parenteral Agents. Measurement of *in vitro* activity against *Pseudomonas aeruginosa* of the investigational cephalosporin class antibiotic cefsulodin sodium (Abbott-46811) was discontinued during this reporting period in favor of new licensed cephalosporins which display similar activity against *Pseudomonas aeruginosa* as cefsulodin sodium but with a broader spectrum of activity. The *in vitro* activities of these newly approved antibiotics are presented in Table 1. A clinical trial of ceftazidime as monotherapy for infected burn patients is in progress. Cefriaxone and aztreonam have not been clinically used to date.

TABLE 1. Activity of Experimental Antibiotics for Fiscal Year 1988

	Ceftazidime[a]	Ceftriaxone[a]	Aztreonam[b]
Resistant	480 (15.4%)	843 (37.0%)	540 (33.9%)
Sensitive	2,633	2,279	1,593

( ) = Percent resistant. [a]Against all flora except oxacillin-resistant *Staphylococcus aureus*. [b]Against Gram-negative aerobic flora.

Cross resistance to all tested cephalosporins has been observed. The mechanism of resistance appears to be a newly described beta-lactamase enzyme (1). Examination of resistant strains by isoelectric-focusing techniques has shown them to contain multiple beta-lactamase activities. One activity at an isoelectric point of 6.3 appears to be common among the strains. Investigations into the location (plasmid and/or chromosomal) of the gene responsible for this enzyme are being conducted. The multiply resistant phenotype has been found in 3 enteric Gram-negative species isolated from 7 burn patients.

Sodium hyaluronate (Pharmacia, Inc., Piscataway NJ), a proposed antimicrobial agent, has been tested for antipseudomonal activity in the modified burned rat model. Two concentrations of this compound were tested, with treatment immediately and 5 days postburn. Rats were inoculated with *Pseudomonas aeruginosa* (Strain 59-1244) on the fifth postburn day. A nontherapeutic protein, ovalbumin, was used as the control. The results are summarized in Table 2.

Experimental Topical Agents. Five-percent mafenide acetate was examined for *in vitro* activity against *Pseudomonas*

TABLE 2A. Examination of Sodium Hyaluronate (0.05%) in *Pseudomonas aeruginosa*-Infected Rats (Dead/Total)

Group	1st Study	2nd Study	Total	Mortality (%)
Control	21/23	9/25	30/48	63
Treatment	22/24	21/24	43/48	90

TABLE 2B. Examination of Sodium Hyaluronate (0.1%) in *Pseudomonas aeruginosa*-Infected Rats (Dead/Total)

Group	1st Study	2nd Study	Total	Mortality (%)
Control	10/25	3/25	13/50	26
Treatment	11/25	2/25	13/50	26

aeruginosa isolated from 61 burn patients. Agar dilution minimal inhibitory concentration (MIC) assays were done on 158 strains. The mean MIC was 0.212 g/100 ml. The median MIC was 0.156 g/100 ml. Data comparing fiscal years 1987 and 1988 are presented in Table 3.

Serologic Types of *Pseudomonas aeruginosa* Isolated from Burn Patients. *Pseudomonas aeruginosa* isolates from 61 patients were serotyped using the Difco International Typing Sera™ set and autoclaved bacterial suspensions. Strains were selected on the basis of having a distinct antibiotic sensitivity pattern for each patient. A total of 158 strains were typed. Data are presented as the total number of patients with each serotype and the total number of isolates per serotype in Figure 1. Serotypes 01, 04, and 11 were the predominant types identified.

Modification of Animal Model. To better simulate a clinical infection in the burned animal model, inoculation of the burned rat was delayed 5-10 days postburn. The value of keeping the wound moist prior to inoculation was evaluated (Table 4). The wounds were covered with a silver-nylon/velfoam dressing and moistened daily with sterile water until inoculated with *Pseudomonas aeruginosa* (Strain 59-1244). The dressing was later modified by the addition of a rigid support and cannula between the silver-nylon and velfoam to improve irrigation (Table 5). Time studies (Table 6) and titration of

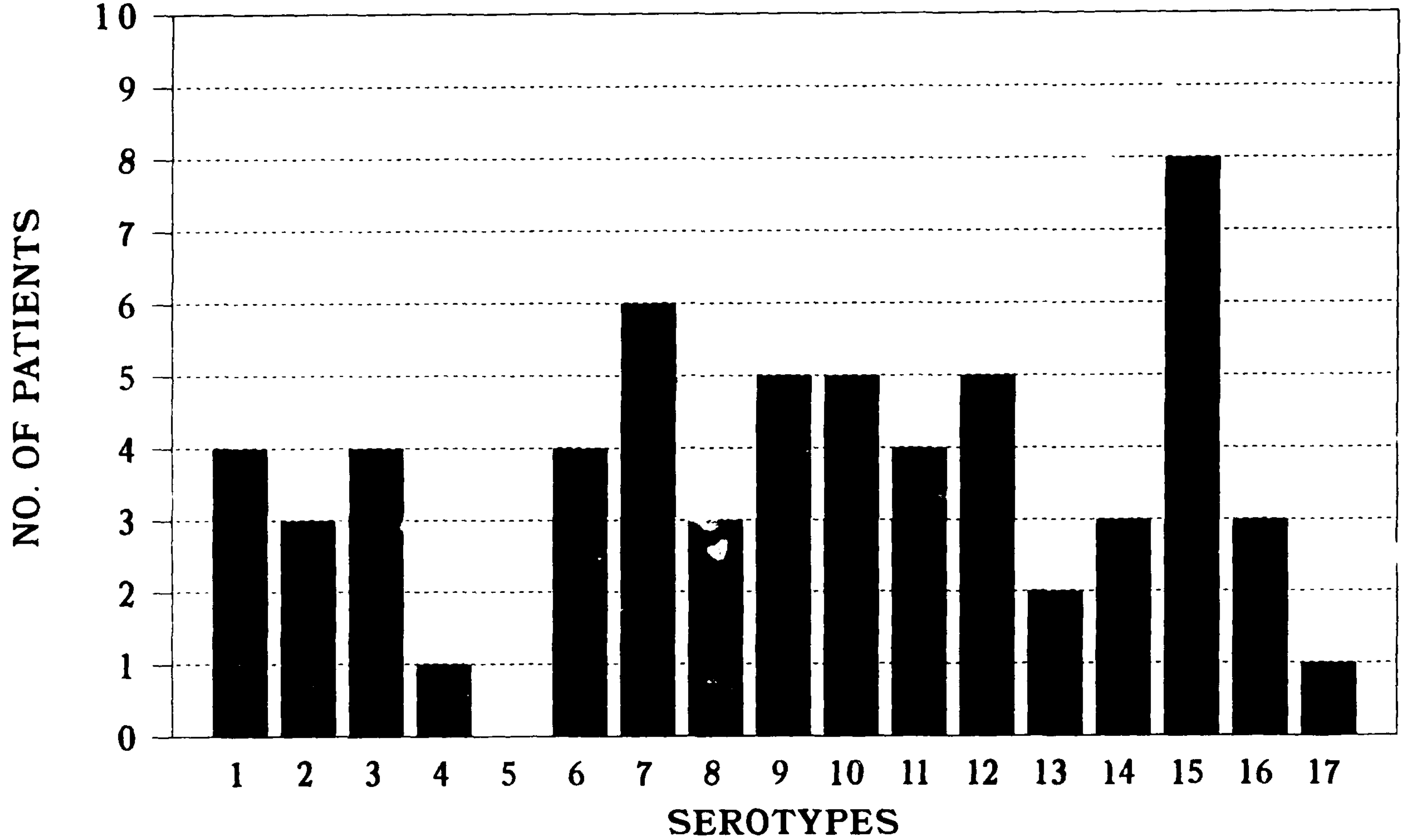

FIGURE 1. Frequency of Pseudomonas serotypes.

TABLE 3. Minimal Inhibitory Concentration for *Pseudomonas aeruginosa* Strains to Mafenide Acetate

Mafenide Acetate Concentration (g/100 ml)	**Number of Strains Fiscal Year 1987**	**Number of Strains Fiscal Year 1988**
0.019	1	10
0.039	5	16
0.078	12	36
0.156	21	39
0.312	21	42
0.625	19	13
1.250	-	2
Total Number of Strains	**79**	**158**

TABLE 4. Effect of Moist Dressing at 0, 5, and 10 Days Following Inoculation (Dead/Total)

	1st Study	2nd Study	3rd Study	Mortality (%)
Day 0	10/10	10/10	10/10	100
Day 5				
+ Dressing	20/20	9/9	5/5	100
- Dressing	12/17	8/10	6/10	70
Day 10				
+ Dressing	-	0/6	1/5	9
- Dressing	-	0/10	1/10	5

the Pseudomonas (Table 7) were carried out to determine the optimum time of inoculation and optimum concentration of Pseudomonas to use to establish an LD100. These delayed infection studies showed that it is possible to infect burned rats several days after burn injury, although on a limited basis.

TABLE 5. Flexible vs. Rigid Dressing 5 Days Following Inoculation with Pseudomonas aeruginosa (Dead/Total)

	- Infection/ - Dressing	+ Infection/ - Dressing	Flexible Dressing	Rigid Dressing
1st study	10/10	14/20	5/5	2/2
2nd study	9/10	1/19	3/8	3/5
Mortality (%)	95	38	62	71

TABLE 6. Time Study with Pseudomonas aeruginosa Inoculation (Dead/Total)

Postburn Day of Inoculation	5	6	7	8	9
1st study	0/11	0/10	1/11	0/11	1/11
2nd study	5/11	1/11	0/11	0/10	0/10
Mortality (%)	23	5	5	0	5

TABLE 7. Titration of Pseudomonas aeruginosa lethal dose at 5 Days Postburn

cfu/ml	10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
1st study	0/5	0/5	0/5	0/6	1/6	1/6	4/6	3/6	2/5	3/5
2nd study	0/5	0/5	0/5	1/6	0/6	1/6	0/6	1/6	3/5	0/5
Mortality (%)	0	0	0	8	8	17	33	33	50	30

The delayed infection model was also tested with a highly virulent strain of Proteus mirabilis (Table 8). The Proteus was less effective in establishing an infection than Pseudomonas.

TABLE 8. Titration of *Proteus mirabilis* lethal dose at 5 Days Postburn

cfu/ml	10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
Live/Dead	0/5	0/5	0/5	0/5	2/5	1/6	1/6	1/6	1/6	0/6
Mortality (%)	0	0	0	0	40	17	17	17	17	0

PRESENTATIONS

McManus AT: Oral nystatin does not alter the incidence of Candidemia in severely burned patients. Presented at the 20th Annual Meeting of the American Burn Association, Seattle, Washington, 25 March 1988.

PUBLICATIONS

McManus WF, McManus AT, Graves TA, and Pruitt BA Jr: Appendix 36-1: Characteristics of commonly used antimicrobial agents. In Davis JH, Drucker WR, Foster RS Jr, Gamelli RL, Gann DS, Pruitt BA Jr, and Sheldon GF (eds), **Clinical Surgery.** St. Louis: CV Mosby Co, 1987, Vol II, pp 944-69.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS: Characterization of Biochemical Indicators of Infection in the Thermally Injured

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David G. Burleson, PhD, Lieutenant Colonel, MS
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Basil A. Pruitt, Jr., MD, Colonel, MC

We have analyzed the prevalence of a biochemical substance in the blood of burned patients that has an identical HPLC retention time to neopterin. This substance was first discovered in the blood of burned-infected rats and was subsequently found in the blood of some burned patients. The previously described factor, with maximum emission at 420 nm and 355 nm excitation, consists of several fluorescent substances that are resolved by HPLC. The partially purified factor was chromatographically and spectrally similar to neopterin, yet its identity could not be verified by thermospray mass spectrometry. Larger quantities of more highly purified material may be necessary to establish the identity of these fluorescent factors.

CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

Infection poses a serious threat to all severely burned patients and is a continuous obstacle to effective therapy. Timely diagnosis of sepsis can be critical to administration of therapy and patient survival. The metabolic changes induced by burn injury hamper the detection of sepsis and make objective diagnosis more difficult. Abnormal levels of hormones (1,2), acute-phase proteins in serum (3,4), and fluorescent substances (5,6) in blood and plasma have been associated with the presence of inflammation and/or infection in human burn patients and animal burn models. The presence of these substances in blood and plasma likely reflect the metabolic response that the stress of trauma and infection place on the host. A more specific measure of invading pathogens would be desirable, but the spectrum of microbes that would have to be detected at the low levels present in blood early after infection limit the general applicability of microbe-specific biochemical indicators. A biochemical measurement of specific metabolic products of the immune response against an infection challenge would have the advantage of differentiating the metabolic response induced by trauma from the metabolic response induced by activation of the immune system. Such a test might alert the clinician to a potential problem in a more timely and objective manner than currently available.

Several attempts are ongoing to find clinically useful indicators to use as an adjunct to standard microbiological methods of assessing the presence of sepsis (5-7). We have purified and attempted further characterization of the nature of the previously reported fluorescent substances found in the blood of burn victims.

MATERIALS AND METHODS

Measurement of Fluorescent Indicators. One milliliter of anticoagulated blood was mixed with 4 ml of cold (4°C) PCA (0.8 M). After incubation for 10 min, the mixture was centrifuged at 4°C for 10 min at 3000 g. The supernatant was recentrifuged at 20000 g for 30 min. The clear supernatant was transferred to another tube and fluorescence was then measured using a spectrofluorometer (SLM Instruments, Inc., Urbana IL) at excitation 355 nm/emission 420 nm (355ex/420em). The fluorometer was standardized by using a calibration standard (fluorescence intensity block).

HPLC Determination of the 355ex/420em Factor in Serum. Serum (100 μl) was deproteinized by incubating at 100°C in an oil bath for 20 min after the addition of 200 μl of 0.2 M potassium phosphate buffer (pH 4.5). The mixture was then centrifuged at 20000 g for 20 min and the supernatant was

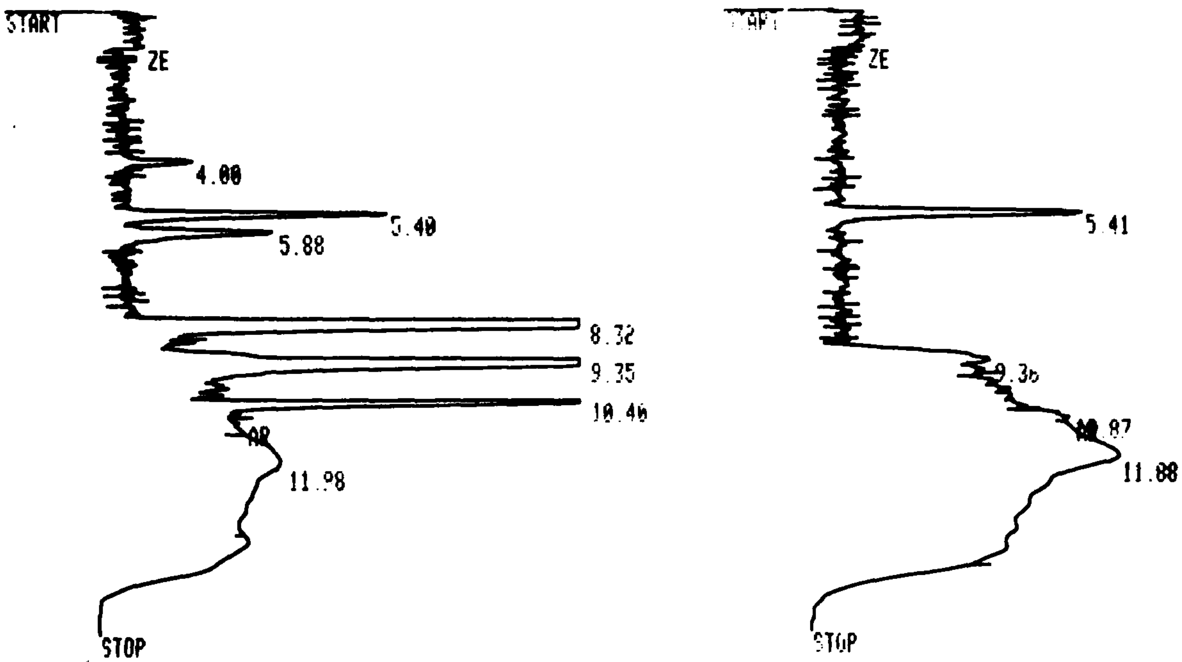
injected directly on HPLC. HPLC was performed on an Hewlett-Packard liquid chromatograph (Model 1090) with a Biophase ODS reverse phase 4.6 X 250 mm column (Bioanalytical Systems, Inc., West Lafayette IN). The mobile phase consisted of 0.05 M ammonium acetate at pH 7.0. The column temperature was maintained at 45°C and the flow rate was 1.0 ml/min. The HPLC was equipped with a Kratos fluorescence detector (Model 980) with a 25-µl flow cell. The excitation monochronometer was set at 350 nm and the emission cutoff filter was at 389 nm. The retention time for standard pterins (Sigma® Chemical Company, St. Louis MO) was determined using 10 µl of a standard solution of pterins (10 ng/ml). The amount of each fluorescent substance present was measured on a Hewlett-Packard integrator (Model 3392A).

Mass Spectral Analysis of the 355ex/420em Factor. Mass spectral analysis of the ion exchange purified material was attempted by derivatization of the purified HPLC effluent to make it volatile enough for gas chromatography so it could be introduced into the mass spectrometer. The mass spectral analysis was attempted on a Hewlett-Packard Model 5985. The gas-liquid chromatography column was a packed column with OU-17 as the liquid phase, the flow rate was 30 ml/min, and the gas phase was helium.

RESULTS

The optimal chromatographic conditions described in the MATERIALS AND METHODS section were determined for chromatography of the 355ex/420em factor and several pterins. The chromatogram obtained for the pterins under these conditions is shown in Figure 1. Neopterin had a retention time of 5.4 min, iso-xanthopterin 8.3 min, and biopterin 9.4 min under these conditions. A patient sample which registered a relatively high fluorescence at 355ex/420em was deproteinized and run on HPLC. The chromatogram obtained is compared with a sample extracted from a normal control in Figure 2. Three of the peaks in the patient sample had retention times similar to neopterin, iso-xanthropterin, and biopterin, respectively (Fig 1) while none of the same peaks were present in the control sample.

To further characterize the nature of the unknown material, 15 ml of human burn patient serum was pooled and the serum protein denatured by heat. The fluorescent material remaining in the supernatant after centrifugation was purified using a modified ion exchange procedure for the purification of pterins. An HPLC chromatogram of the purified serum components is compared to unpurified serum in Figure 3. The peak integral value for each of the peaks remaining after purification were compared to the peak integral value of fluorescent material present before purification. The peak at 5.4 min was selectively purified by 7.2-fold compared to the total

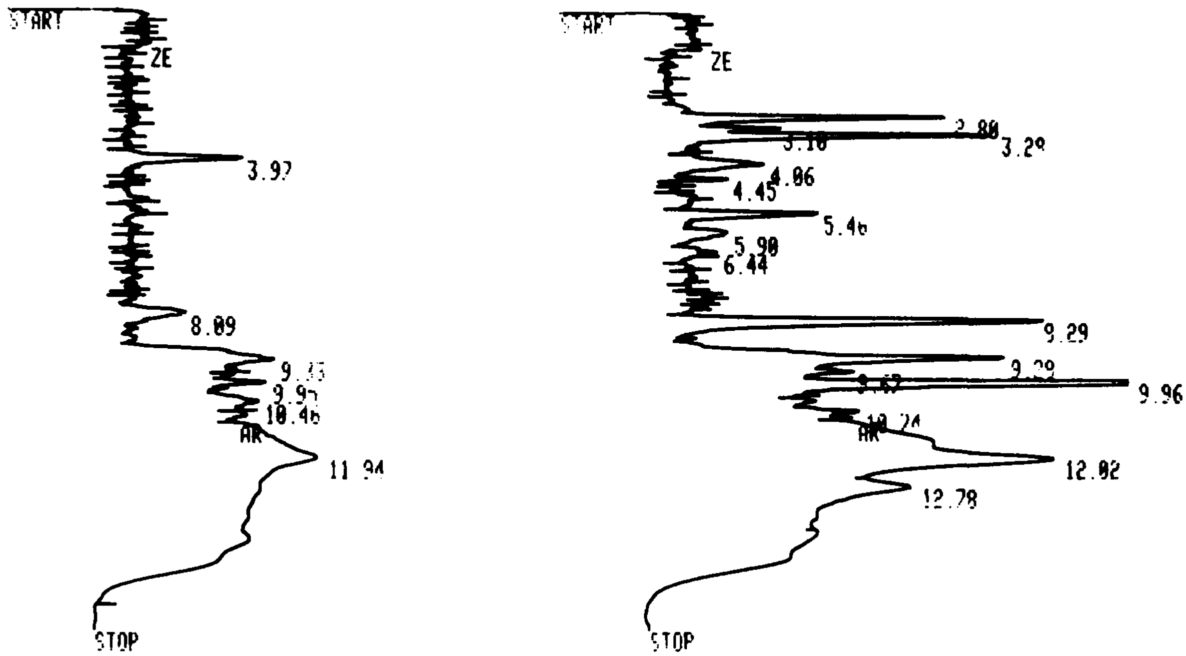


A B

FIGURE 1. Chromatogram of neopterin and five other pterins. **A.** Neopterin standard alone. **B.** Neopterin and 5 other pterins. A 5 μl aliquot of a standard solution containing 10 μg/ml of pterin-6-carboxylic acid (4.0 min), neopterin (5.4 min), xanthropterin (5.88 min), iso-xanthopterin, biopterin (9.35 min), and 6-methyl pterin (10.4 min) was chromatographed under the conditions described in the text.

fluorescence in the sample. The peaks at 9.3 and 9.95 min retained their relative concentrations after purification. The amount of the 5.4-min material present correlated to 0.948 μg/ml of neopterin standard.

Mass spectral analysis was performed on the partially purified sample in order to try to verify the identity of the peak at 5.4 min. Although the optical and fluorescence spectral characteristics, chemical behavior, and HPLC of this material were similar to that of neopterin, confirmation of the identity as neopterin by comparison of the unknown peak with that of a known standard was unsuccessful. Since there is no fluorescence detection on the mass spectrometer, substances eluting from the HPLC column were detected by monitoring the total ion fragments produced in the ionization chamber of the mass spectrometer (Fig 4). There was relatively little material detected by total ion count monitoring at the retention time corresponding to neopterin. The amount of



A **B**

FIGURE 2. Comparison of chromatograms from a highly fluorescent patient sample and a normal control. **A.** Patient sample. **B.** Control sample. Chromatography conditions were as described in the text.

unknown introduced into the mass spectrometer (based on fluorescence equivalence) was equal to 2 ng of neopterin, 2% of that shown in Figure 4B. This level was close to the lower detection limit for neopterin. The presence of so much nonfluorescent material may have obscured the presence of neopterin in the patient sample. Specific monitoring for the parent ion of neopterin (m+1, 254 amu) and the principal ion fragments (192 and 218 amu) also failed to reveal the presence of neopterin (Fig 5). Mass spectrometry analysis did reveal a substance of molecular weight of 186 amu at 0.5 min before the retention time of the neopterin standard. A chemical composition of the 186 amu peak could not be obtained due to the high background levels in the sample which prevented the determination of an accurate isotopic abundance ratio for the m+2 and m+3 peak. Since HPLC columns can vary slightly, it is not clear whether the 186 amu peak is the same as that corresponding with a neopterin retention time in HPLC with fluorescence detection. As is common with thermospray techniques, the limited fragmentation pattern revealed little other structural clues to the structure of the 186 amu peak.

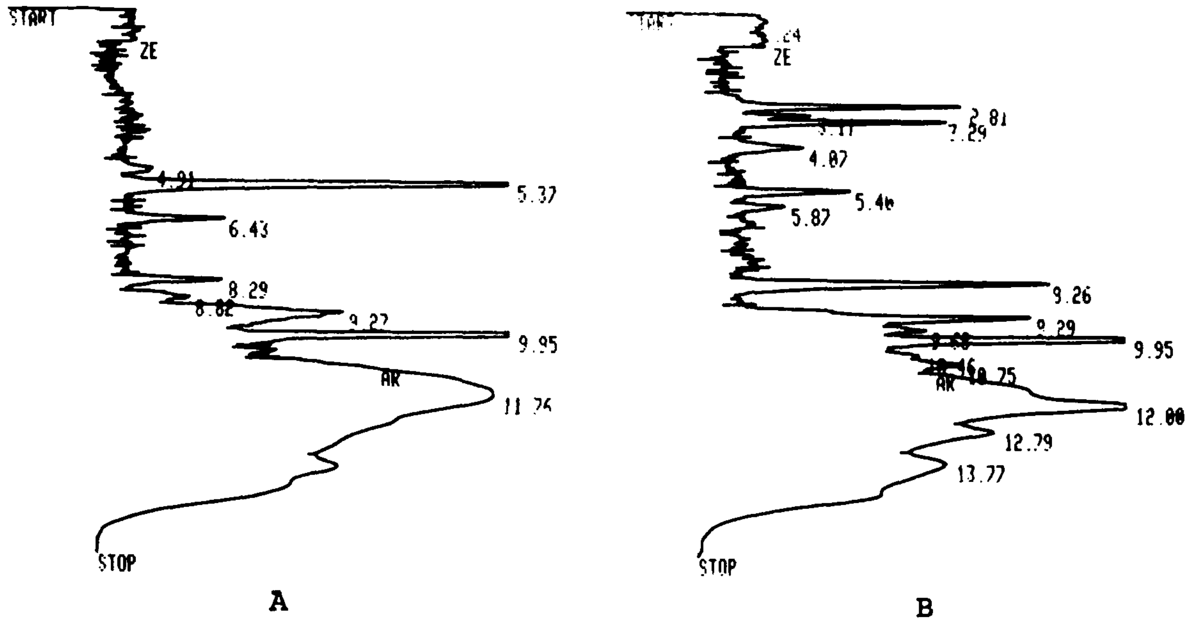


FIGURE 3. Comparison of pooled fluorescent patient sera before and after purification by ion exchange chromatography. **A.** Purified sample eluted from ion exchange column. **B.** Pooled sera before purification. A pool of several patient sera with a high level of 355ex/420em factor was purified as described in the text. A portion of the pooled sera was chromatographed before and after purification for comparison.

In addition to the fluorescent peak with a retention time similar to neopterin, three other fluorescent substances are present consistently when the PCA supernatant is highly fluorescent at 355ex/420em. The chromatogram for several patients with high levels of 355ex/420em factor are shown in Figure 6. Neopterin had a retention time of 5.3 min under the conditions employed when these chromatograms were obtained. Three other fluorescent substances were consistently present at retention times at approximately 8.2, 9.2, and 9.9 min. The combination of the four peaks constituted an average of 72.1% of the fluorescence measured during the first 10 min of analysis by HPLC under these conditions.

DISCUSSION

We have separated four components that are consistently found in extracts of patient serum that have highly fluorescence PCA filtrates. These substances have fluorescent spectral characteristics similar to nucleotide derivatives such as the pterins. Three of the components copurify with and behave chromatographically similar to pterins, yet we have not

A

B

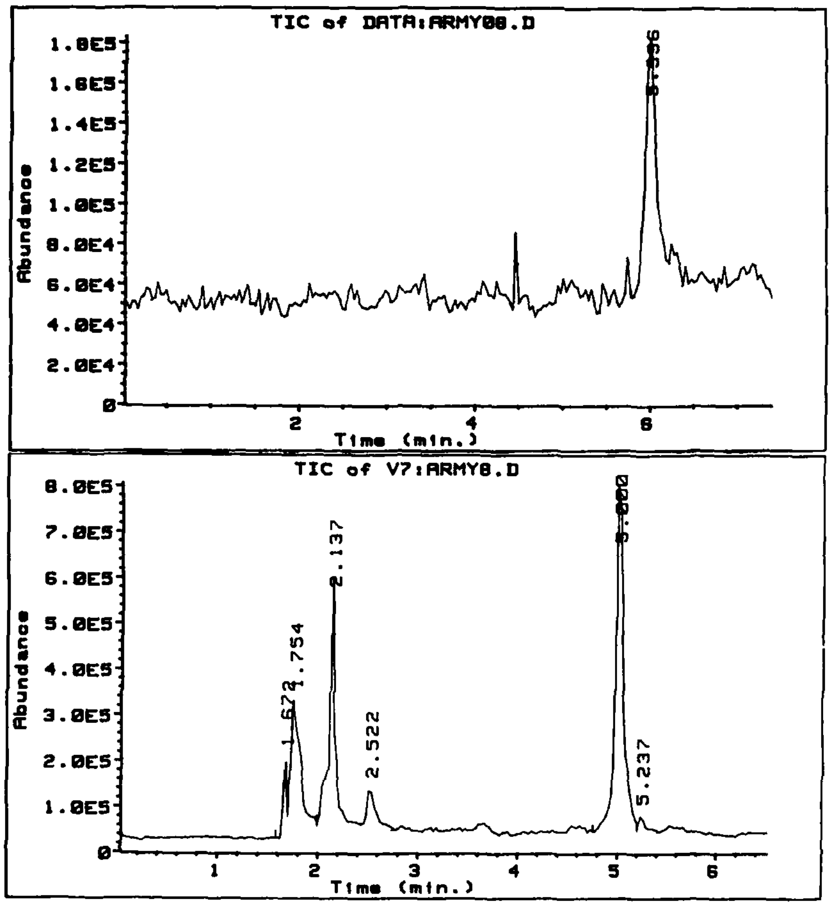


FIGURE 4. Comparison of chromatograms of purified patient sera and standard neopterin using mass spectral total ion count (TIC) for sample detection. **A.** Partially purified patient sample. **B.** Neopterin standard. The partially purified patient sample was chromatographed by HPLC and the effluent monitored with a mass spectrometer. TIC of each substance emitted from the column is shown relative to the TIC of 100 ng standard neopterin chromatographed under the same conditions. Note the relatively small amount of material with a retention time in the range of neopterin.

established chemical identity with any of the commonly found pterin derivatives to which they have been compared.

It is difficult to accurately determine how much of the fluorescence seen in the original PCA supernatant extracts from patient serum can be accounted for by these four chromatographically separated substances. Fluorescence is highly dependent on chemical structure as well as environmental

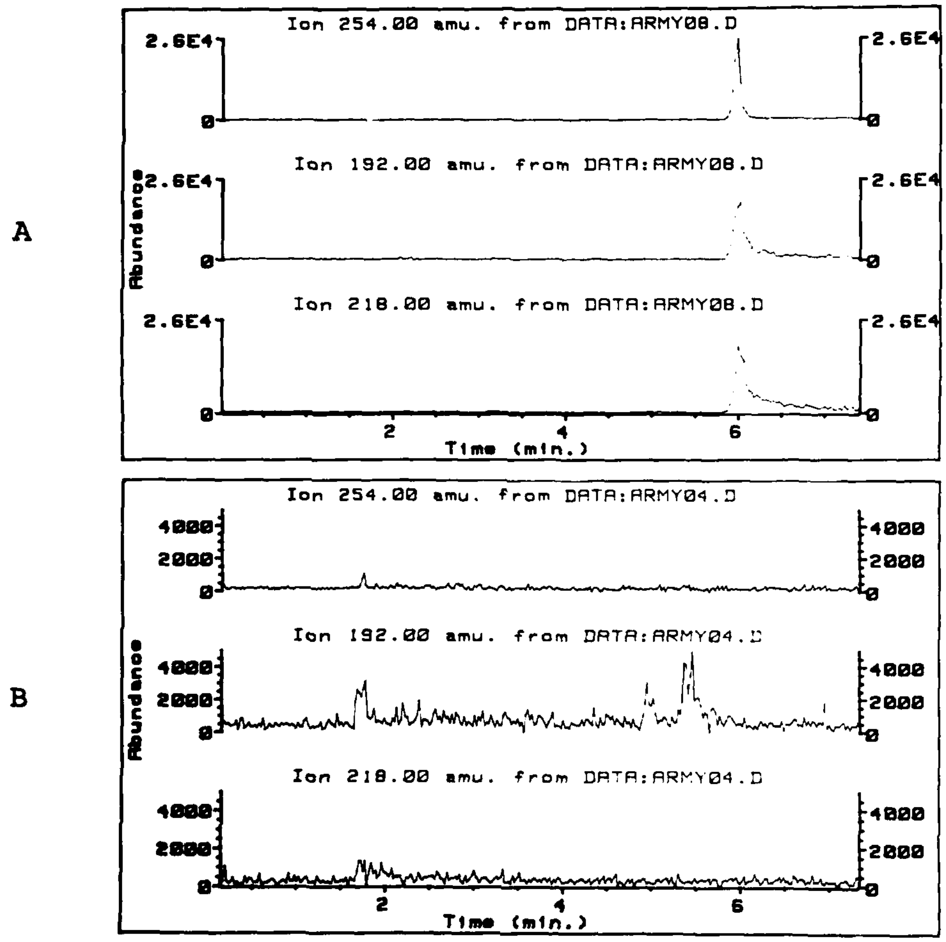


FIGURE 5. Comparison of HPLC chromatograms of partially purified patient sera and standard neopterin using mass spectral single ion monitoring for detection. **A.** Partially purified patient sample. **B.** Neopterin standard. Effluent from HPLC separation of the ion exchange purified patient sera was directed into the thermosray module of a mass spectrometer. The parent (M+1 = 254 amu) and major fragmentation ions (218 and 192 amu) for standard neopterin were monitored specifically to detect neopterin. The amount of fluorescent material at the neopterin retention time based on calculations of neopterin fluorescence was approximately 2% of the standard shown in the single ion monitor chromatogram. No neopterin specific peaks are visible above background in the purified sera sample.

factors such as pH. Since the pH of the detector chamber is approximately 7.4 and the fluorescent factors were originally

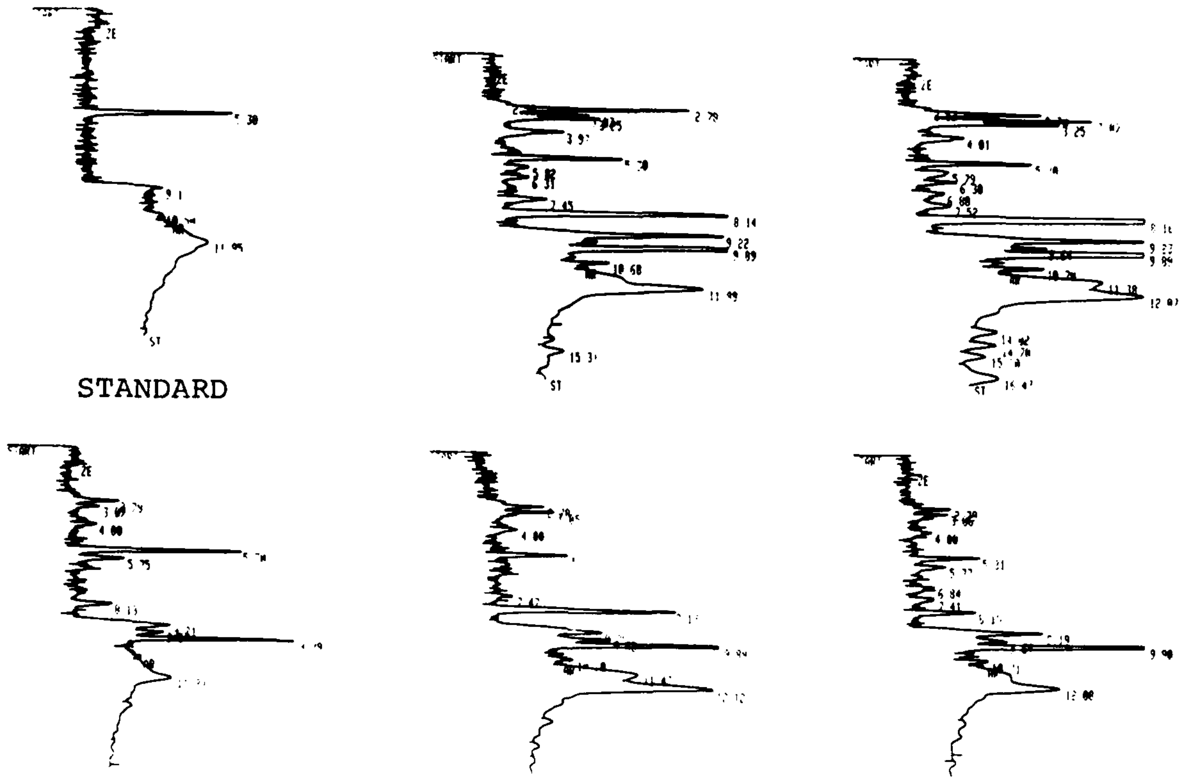


FIGURE 6. Comparison of HPLC chromatograms of patient samples with high fluorescence readings with neopterin standard. Five patient samples with high values for the 355ex/420em factor were chromatographed by HPLC with a fluorescence detector. Peaks are consistently present at 5.3 $\pm$ 0.1, 8.14 $\pm$ 0.2, 9.21 $\pm$ 0.2, and 9.9 $\pm$ 0.2 in each patient sample.

measured in concentrated PCA solution, it is not possible to quantitatively compare the fluorescence. We cannot assume that all of the fluorescent substances present in the supernatant extracts are detectable under the chromatographic conditions employed.

Several attempts have been made to derivatize the material and obtain an ionization fragmentation pattern by GC-MS, which is the most sensitive method of determination of chemical structure available. The suitable derivatives have not been attained to this point.

Further chemical characterization may not be possible on the small amount of material of limited purity that we have obtained so far. Future attempts at identification of these fluorescent substances will be attempted after collection and purification of milligram quantities of these substances.

PRESENTATIONS/PUBLICATIONS

None.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST DEFENSE IN BURNED SOLDIERS: Mafenide Does Not Inhibit Dihydropteroate Synthase

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Robert G. Eagon, PhD*
Albert T. McManus, PhD

*Department of Microbiology
University of Georgia
Athens, Georgia 30602

ABSTRACT

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Using intact cells, it was found that *Pseudomonas aeruginosa* was more susceptible to mafenide than *Escherichia coli*, that para-aminobenzoic acid (PAB) did not reverse or prevent inhibition by mafenide, and that PAB itself was inhibitory. Under the experimental conditions used in these studies, PAB was more inhibitory toward *Escherichia coli* than *Pseudomonas aeruginosa*. It was proposed that PAB might be useful for topical treatment of burn wounds. At the enzyme level, it was shown that mafenide did not exert its inhibitory effects in the same manner as the structually related sulfonamides.

MAFENIDE DOES NOT INHIBIT DIHYDROPTEROATE SYNTHASE

Mafenide, which is also known as Marfanil[R] and as Sulfamylon[R], was first synthesized in the United States around 1938. Its use was abandoned in the United States when it was found to be ineffective against streptococci and to be only feebly active aganist infective microorganisms in general (1,2). During World War II, however, mafenide was issued to German soldiers, particularlly to those on the Eastern front, in powder form for local application to wounds (1). This use of mafenide was apparently successful, particularly for the control of infections caused by anaerobic organisms (2).

Mafenide exerts low bacteriostatic activity against a wide spectum of both Gram-postive and Gram-negative bacteria (3). Mafenide, however, is particularly effective against <u>Pseudomonas aeruginosa</u> (3-5), a troublesome organism in burn wound tissues. Indeed, the first extensive use of mafenide was pioneered by the Institute to treat burn wound infections (6,7). Moreover, this remains the primary use of mafenide today (3-6).

Mafenide is a synthetic antimicrobial agent structurally related to the sulfonamides as well as to para-aminobenzoic acid (PAB). Chemically, mafenide is para-aminomethylbenzene sulfonamide. It is most commonly used as the acetate salt.

The structure of mafenide differs from that of the sulfonamides in that it has an aminomethyl group in the <u>para</u> position on the benzene ring instead of an unsubstituted <u>amino</u> group. It would be expected, nevertheless, that the mechanism of action of mafenide would be the same as that of the sulfonamides. The sulfonamides, being structural analogs of PAB, compete with PAB for the same binding site on dihyropteroate synthase (EC 2.5.1.15). In so doing, they interfere directly with the synthesis of dihydropteroic acid, a precursor of tetrahydrofolic acid, the coenzymatic form of folic acid. Specifically, dihydropteroic acid is synthesized as follows (8,9):

2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine-pyrophospate + para-aminobenzoate

$$\Big\downarrow \; Mg^{2+} \quad \text{dihydropteroate synthase}$$

dihydropteroate + pyrophosphate

Surprisingly, the mechanism of action of mafenide is considered to differ from that of the sulfonamides (2-6). The concept that the mode of action of mafenide differs from that of the sulfonamies was based on the indirect observations,

i.e., that the inhibitory action of mafenide was not antagonized by PAB, serum, pus, or tissue exudates and that there was no correlation between bacterial sensitivities to mafenide and to the sulfonamides.

A search of the literature revealed no published data at the enzyme level to support the supposition that the mechanism of action of mafenide was indeed different from that of the sulfonamides. With the foregoing in mind, the purpose of the study described herein was to measure directly the effect of mafenide on dihydrodropteroate synthase.

MATERIALS AND METHODS

Organisms. The two experimental organisms used in these studies were *Escherichia coli*, a clinical isolate from a urine specimen, and *Pseudomonas aeruginosa* (ATCC 27317). The latter organism is considered to be the "standard virulent strain" of *Pseudomonas aeruginosa* and is also referred to as Strain 1244.

Media. Unless otherwise indicated, the organisms were grown in a chemically defined basal salts medium (10) supplemented, in final concentration, with 20 mM glucose (BSG).

Minimal Inhibitory Concentration (MIC) Determinations. The tube serial dilution technique was used to determine MIC using BSG as the test medium.

Preparation of Cell-Free Extracts. Cells of *Pseudomonas aeruginosa* and *Escherichia coli* were grown overnight at 37°C in trypticase soy broth (BBL Microbiology Systems, Cookeysville MD) on a reciprocating shaker. The cells were harvested by centrifuging, washed once with 10 mM phosphate buffer (pH 7), and then suspended in two volumes of the same buffer. The cells were ruptured by two passes through a French pressure cell at 18,000 psi. DNase and RNase were added (final concentration, 100 μg/ml) to the broken cell suspensions. Intact cells were removed by centrifuging at 36,000 g for 30 min at 4°C. The supernatant was then centrifuged at 100,000 g for 1 h at 4°C.

The supernatant from the latter centrifugation was brought to 55% saturation with $(NH_4)_2SO_4$ by drop-wise addition of a saturated $(NH_4)_2SO_4$ solution at 0-4°C. The precipitated proteins were sedimented by centrifuging at 36,000 g for 45 min at 4°C. The sediments were then dissolved in 10 mM phosphate buffer (pH 7) and dialyzed with gentle agitation for 24 h against the same buffer at 0-4°C. The extracts were stored in 0.5-ml samples at -70°C until needed.

Protein concentrations were determined by the Coomasie blue technique using the Bio-Rad protein assay kit (Bio-Rad

Laboratories, Richmond CA). Bovine serum albumin was used as the standard.

Assay for the Effect of Various Agents on Dihydropteroate Synthase. In a total volume of 200 μl, the following reagents, in final concentration, were used to measure the activity of dihydropteroate synthase: Tris-HCl buffer (pH 8.3), 40 mM; $MgCl_2$, 5mM; dithiothreitol, 5 mM; 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine-pyrophosphate (H_2PtCH_2OPP), 20 μM; [ring-UL-^{14}C]PAB, 20 μM; cell extract, 0.5 mg protein/ml; and mafenide-HCl, sodium sulfadiazine, or KCl in final concentration (see Tables 1-3).

The reactions were incubated for 5 min in 1.5-ml microfuge tubes in a 37°C water bath. The reactions were started by the addition of the cell extracts and were stopped by placing the incubation tubes in ice.

A 100-μl sample from each reaction was added to an area of 2 X 3 cm on Whatman 3MM chromatography paper. Ascending chromatography was run in 0.1 M phosphate buffer (pH 7). After developing, the origins, i.e., 2 X 3-cm areas, were cut out, placed in minivials with 6 ml of Packard Opti-fluor scintillation fluid (Packard Instrument Company, Inc., Downers Grove IL), and their radioactivities determined. In this procedure, the ^{14}C-labeled dihydropteroate remained at the origin while the unreacted ^{14}C-PAB migrated with an R_f = 0.8.

Reagents. DNase, RNase, and [ring-UL-^{14}C]PAB were purchased from the Sigma Chemical Company (St. Louis MO). Phosphanilic acid was purchased from Raylo Chemicals (Edmonton, Alberta, Canada). All other reagents were purchased from commercial sources in their highest state of purity.

RESULTS

MIC of Mafenide, Sulfanilamide, and PAB for *Pseudomonas aeruginosa* and *Escherichia coli* in BSG Medium. The MIC procedure used in these studies should not be confused with the standardized procedures using enriched Mueller-Hinton medium. Instead, the minimal medium, BSG, was used to avoid the presence of intermediates and end products of folic acid metabolism which might mask the inhibitory effects of weak inhibitors of dihydropteroate synthesis. Using this procedure, *Pseudomonas aeruginosa* was more susceptible to inhibition by mafenide than *Escherichia coli* (see Table 2). This observation paralleled clinical evidence (unpublished observations).

Sulfanilamide was used as a control because it is known to be a weak inhibitor of dihydropteroate synthesis, i.e., sulfanilamide is weakly competitive with PAB. A comparison of the inhibition of *Pseudomonas aeruginosa* by mafenide with that by sulfanilamide showed that twice the concentration of

TABLE 1. Minimal Inhibitory Concentrations (MIC) of Mafenide and Sulfanilamide in the Absence and Presence of Para-Aminobenzoate (PAB) and MIC of PAB alone for _Pseudomonas aeruginosa_ and _Escherichia coli_ in Basal Salts Supplemented with 20 mM Glucose (BSG)

Agent	MIC (μg/ml)[a] _Pseudomonas aeruginosa_[b]	_Escherichia coli_[c]
Sulfanilamide	200 (600)	200
Sulfanilamide + 200 μg/ml PAB	> 1,000[d]	400
Mafenide	400 (> 1,000[d])	> 1,000[d]
Mafenide + 5 μg/ml PAB	600 (> 1,000[d])	NOT DONE
Mafenide + 10 μg/ml PAB	500 (> 1,000[d])	NOT DONE
Mafenide + 100 μg/ml PAB	400 (> 1,000[d])	NOT DONE
Mafenide + 200 μg/ml PAB	400 (> 1,000[d])	> 1,000[d]
PAB	500 (500)	200

[a] The tube serial dilution technique was used in which the concentration of the agents was increased by increments of 100 μg/ml from 100 to 1,000 μg/ml or by increments of μg/ml from 10 to 100 μg/ml.
[b] The nonparenthetical numbers were results afer 24 h incubation while the parenthetical numbers were results after 48 h incubation.
[c] _Escherichia coli_ grew slowly on BSG and turbidity did not occur until in excess of 24 h incubation. Thus, the results shown here represent 48-72 h incubation.
[d] Concentrations > 1,000 μg/ml were not used.

mafenide as sulfanilamide was required to inhibit this organism (Table 1). Upon continued inhibition, sulfanilamide remained the better inhibitor. Mafenide was less effective against _Escherichia coli_ than _Pseudomonas aeruginosa_ whereas sulfanilamide was about equally effective against _Escherichia coli_ as against _Pseudomonas aeruginosa_.

PAB was not effective in overcoming the inhibitory effects of mafenide against _Pseudomonas aeruginosa_, irrespective of the concentration of PAB (Table 1). Surprisingly, PAB itself was inhibitory to both _Pseudomonas aeruginosa_ and _Escherichia coli_

TABLE 2. Effect of Mafenide on Dihydropteroate Synthase of *Pseudomonas aeruginosa* and *Escherichia coli*

	Pseudomonas aeruginosa		*Escherichia coli*	
	Enzyme Activity[a]	% Inhibition	Enzyme Activity[a]	% Inhibition
0.02 mM PAB	0.209; 0.216[b]		0.136	
0.02 mM PAB + 0.2 mM sulfadiazine	0	100	0	100
0.02 mM PAB + 0.2 mM mafenide	0.200	4	NOT DONE	
0.02 mM PAB + 2 mM mafenide	0.200	4	NOT DONE	
0.02 mM PAB + 20 mM mafenide	0.137	37	0.103	100

[a] Enzyme activity = nmol dihydropteroate produced min^{-1} mg $protein^{-1}$.
[b] The two enzyme activity base values cited were derived from separate experiments. The % inhibition values were calculated using the base value which was appropriate for that particular experiment.

TABLE 3. Effect of KCl on Dihydropteroate Synthase of *Pseudomonas aeruginosa*

	Enzyme Activity[a]	% Inhibition
0.02 mM PAB	0.214	
0.02 mM PAB + 200 mM KCl	0.191	11
0.02 mM PAB + 601.35 mM KCl	0.083	61

[a]Enzyme activity = nmol dihydropteroate produced min^{-1} mg $protein^{-1}$.

under these experimental conditions. Thus, a concentration of 500 μg/ml of PAB inhibited *Pseudomonas aeruginosa* in a 24-h incubation period contrasted to 400 μg/ml of mafenide.

Effect of Mafenide and KCl on Dihydropteroate Synthase. Mafenide did not inhibit the synthesis of dihydropteroate by dihydropteroate synthase in extracts of either *Pseudomonas aeruginosa* and *Escherichia coli* when used in mafenide:PAB ratios (mol:mol) of 100:1 and 1,000:1 (Table 2). When a mol:mol ratio of 10,000:1 of mafenide:PAB was used, however, 37% and 24% inhibition of dihydropteroate synthase from *Pseudomonas aeruginosa* and *Escherichia coli*, respectively, was noted.

As a control, sulfadiazine, which is a well-documented inhibitor of dihydropteroate synthesis, was used. Thus, at a mol:mol ratio of 10:1 of sulfadiazine:PAB, sulfadiazine completely inhibited the synthesis of dihydropteroate by extracts of both *Pseudomonas aeruginosa* and *Escherichia coli* (Table 2).

A 200 mM solution of mafenide, which is a 10,000:1 (mol:mol) ratio with PAB, is a 4.45% solution (wt:vol). To determine whether the inhibition of dihydropteroate synthesis by mafenide at this concentration was due to a "salt effect" on the synthase, KCl was substituted for mafenide. The results are shown in Table 3. A 10,000:1 (mol:mol) ratio of KCl:PAB resulted in an 11% inhibition of dihydropteroate synthesis by an extract of *Pseudomonas aeruginosa*, but a 4.45% solution (wt:vol) of KCl, i.e., 601.35 mM KCl, resulted in 61% inhibition. From this we concluded that the inhibition of dihydropteroate synthesis by the same high percentage concentration of mafenide was due to a salt effect on dihydropteroate synthase and not to competition with PAB.

DISCUSSION

At the intact cell level, our data showed that *Pseudomonas aeruginosa* was more susceptible to the inhibitory effects of mafenide than *Escherichia coli*, thus confirming past clinical observations that PAB did not reverse or prevent the inhibitory effects of mafenide and that PAB itself was inhibitory.

In the case of the latter observation, PAB was found to be more inhibitory toward *Escherichia coli* than *Pseudomonas aeruginosa*. Under conditions of this study, the MIC of PAB in BSG medium was 200 μg/ml for *Escherichia coli* and 500 μg/ml for *Pseudomonas aeruginosa*. Significantly, PAB was more effective against *Escherichia coli* under our study conditions than mafenide.

The inhibitory concentrations PAB would be high for systemically administered chemotherapeutic purposes. Nevertheless, it seems probable that PAB would be useful for topical application, such as on a burn wound. Our data, however, did not permit us to determine the mechanism of antimicrobial action of PAB.

At the enzyme level, our results confirmed that mafenide did not inhibit dihydropteroate synthase by acting as a competitor of PAB. Thus, although structurally similar to sulfonamides, mafenide did not exert its inhibitory effect in the same manner as the sulfonamides. Our data, however, did not permit us to discern the actual mechanism of antimicrobial action of mafenide.

PRESENTATIONS/PUBLICATIONS

None.

ACKNOWLEDGEMENTS

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ANNUAL RESEARCH PROGRESS REPORT

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PROJECT TITLE: ALTERATION OF HOST DEFENSE IN BURNED SOLDIERS: Phosphanilic Acid Inhibits Dihydropteroate Synthase

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1987 - 30 September 1988

INVESTIGATORS

Robert G. Eagon, PhD*
Albert T. McManus, PhD

*Department of Microbiology
University of Georgia
Athens, Georgia 30602

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PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

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Albert T. McManus, PhD

Intact cells of Pseudomonas aeruginosa were more susceptible to phosphanilic acid (PA) than cells of Escherichia coli. Using cell-free extracts, the dihydropteroate synthases of Pseudomonas aeruginosa and Escherichia coli were about equally susceptible to inhibition by PA. These results suggest that cells of Pseudomonas aeruginosa are more permeable to PA than cells of Escherichia coli. Although a weak inhibitor, PA acted on dihydropteroate synthase in the same manner as the sulfonamides with which PA is structurally related. Inhibition of Escherichia coli by PA in a basal salts-glucose medium was prevented by para-aminobenzoic acid (PAB). However, PAB itself exhibited an inhibitory effect.

PHOSPHANILIC ACID INHIBITS DIHYDROPTEROATE SYNTHASE

Phosphanilic acid (PA) is a structural analog of para-aminobenzoic acid (PAB) as well as sulfanilic acid and the sulfonamides. Chemically, PA is para-aminobenzene phosphonate. Thus, it has a phosphate group in the position occupied by a sulfonate group in the sulfa drugs and by a carboxy group in PAB.

The antimicrobial activity of PA, as that of the sulfonamides, has been reported to be antagonized by PAB in intact cells (1-3). The antimicrobial spectum of PA resembled that of the sulfonamide, sulfamethoxazole, but PA had significantly greater activity against *Pseudomonas aeruginosa* (3). However, while PA was effective in treating infections in mice caused by *Pseudomonas aeruginosa*, the therapeutic effectiveness of PA against other organisms was not impressive (3). Nevertheless, PA may have value in treating certain infections involving *Pseudomonas aeruginosa*, particularly surface infections such as those in burn wounds. Moreover, it seems probable that chemical derivatives of PA may have even greater utility. With respect to the latter, it has been reported that silver phosphanilamidopyrimidine had the same activity against *Pseudomonas aeruginosa* in the burned mouse model as silver sulfadiazine (4).

The sulfonamides, being structural analogs of PAB, compete with PAB for the same binding site on dihydropteroate synthase (EC 2.5.1.15). In so doing, they interfere directly with the synthesis of dihydropteroic acid, a precursor of tetrahydrofolic acid, the coenzymatic form of folic acid. Specifically, dihydropteroic acid is synthesized as follows (5,6):

2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine-pyrophospate + para-aminobenzoate

$$\Big\downarrow \; Mg^{2+} \quad \text{dihydropteroate synthase}$$

dihydropteroate + pyrophosphate

Since the antimicrobial activity of PA is antagonized by PAB in intact bacterial cells, PA would be expected to inhibit organisms at the dihydropteroate synthase level. However, no published experimental evidence on the mode of action of PA at the molecular level could be found. Thus, the studies described herein were undertaken to determine, at the enzyme level, whether PA acts against dihydropteroate synthesis.

MATERIALS AND METHODS

Organisms. The two experimental organisms used in these studies were *Escherichia coli*, a clinical isolate from a urine specimen, and *Pseudomonas aeruginosa* (ATCC 27317). The latter organism is considered to be the "standard virulent strain" of *Pseudomonas aeruginosa* and is also referred to as Strain 1244.

Media. Unless otherwise indicated, the organisms were grown in a chemically defined basal salts medium (7) supplemented, in final concentration, with 20 mM glucose (BSG).

Minimal Inhibitory Concentration (MIC) Determinations. The tube serial dilution technique was used to determine MIC using BSG as the test medium.

Preparation of Cell-Free Extracts. Cells of *Pseudomonas aeruginosa* and *Escherichia coli* were grown overnight at 37°C in trypticase soy broth (BBL Microbiology Systems, Cookeysville MD) on a reciprocating shaker. The cells were harvested by centrifuging, washed once with 10 mM phosphate buffer (pH 7), and then suspended in two volumes of the same buffer. The cells were ruptured by two passes through a French pressure cell at 18,000 psi. DNase and RNase were added (final concentration, 100 μg/ml) to the broken cell suspensions. Intact cells were removed by centrifuging at 36,000 g for 30 min at 4°C. The supernatant was then centrifuged at 100,000 g for 1 h at 4°C.

The supernatant from the latter centrifugation was brought to 55% saturation with $(NH_4)_2SO_4$ by drop-wise addition of a saturated $(NH_4)_2SO_4$ solution at 0-4°C. The precipitated proteins were sedimented by centrifuging at 36,000 g for 45 min at 4°C. The sediments were then dissolved in 10 mM phosphate buffer (pH 7) and dialyzed with gentle agitation for 24 h against the same buffer at 0-4°C. The extracts were stored in 0.5-ml samples at -70°C until needed.

Protein concentrations were determined by the Coomasie blue technique using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond CA). Bovine serum albumin was used as the standard.

Assay for the Effect of Various Agents on Dihydropteroate Synthase. In a total volume of 200 μl, the following reagents, in final concentration, were used to measure the activity of dihydropteroate synthase: Tris-HCl buffer (pH 8.3), 40 mM; $MgCl_2$, 5mM; dithiothreitol, 5 mM; 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine-pyrophosphate (H_2PtCH_2OPP), 20 μM; [ring-UL-^{14}C]PAB, 20 μM; cell extract, 0.5 mg protein/ml; and potassium phosphanilate and sodium sulfadiazine in final concentration (see Table 1).

TABLE 1. Effect of Phosphanilic Acid on Dihydropteroate Synthase of *Pseudomonas aeruginosa* and *Escherichia coli*

	Pseudomonas aeruginosa		*Escherichia coli*	
	Enzyme Activity[a]	% Inhibition	Enzyme Activity[a]	% Inhibition
0.02 mM PAB	0.216; 0.247[b]		0.136	
0.02 mM PAB + 0.2 mM sulfadiazine	0	100	0	100
0.02 mM PAB + 0.2 mM phosphanilate	0.244	1	NOT DONE	
0.02 mM PAB + 2 mM phosphanilate	0.193	22	0.098	28
0.02 mM PAB + 20 mM phosphanilate	0.035	84	0	100

[a]Enzyme activity = nmol dihydropteroate produced min^{-1} mg $protein^{-1}$.
[b]The two enzyme activity base values cited were derived from separate experiments. The % inhibition values were calculated using the base value which was appropriate for that particular experiment.

The reactions were incubated for 5 min in 1.5-ml microfuge tubes in a 37°C water bath. The reactions were started by the addition of the cell extracts and were stopped by placing the incubation tubes in ice.

A 100-μl sample from each reaction was added to an area of 2 X 3 cm on Whatman 3MM chromatography paper. Ascending chromatography was run in 0.1 M phosphate buffer (pH 7). After developing, the origins, i.e., 2 X 3-cm areas, were cut out, placed in minivials with 6 ml of Packard Opti-fluor scintillation fluid (Packard Instrument Company, Inc., Downers Grove IL), and their radioactivities determined. In this procedure, the ^{14}C-labeled dihydropteroate remained at the origin while the unreacted ^{14}C-PAB migrated with an R_f = 0.8.

Reagents. DNase, RNase, and [ring-UL-^{14}C]PAB were purchased from the Sigma Chemical Company (St. Louis MO). Phosphanilic acid was purchased from Raylo Chemicals (Edmonton, Alberta, Canada). All other reagents were purchased from commercial sources in their highest state of purity.

RESULTS

MIC of PA, Sulfanilamide, and PAB for *Pseudomonas aeruginosa* and *Escherichia coli* in BSG Medium. The MIC procedure used in these studies should not be confused with the standardized procedures using enriched Mueller-Hinton medium. Instead, the minimal medium, BSG, was used to avoid the presence of intermediates and end products of folic acid metabolism which might mask the inhibitory effects of weak inhibitors of dihydropteroate synthesis. Using this procedure, *Pseudomonas aeruginosa* was more susceptible to inhibition by PA than *Escherichia coli* (see Table 2).

Sulfanilamide was used as a control because it is known to be a weak inhibitor of dihydropteroate synthesis, i.e., sulfanilamide is weakly competitive with PAB. A comparison of the inhibition of *Pseudomonas aeruginosa* by PA with that by sulfanilamide showed that the PA required to inhibit this organism was a tenth that of sulfanilamide (Table 2). PA was less effective against *Escherichia coli* than against *Pseudomonas aeruginosa*, whereas sulfanilamide was about equally effective against *Escherichia coli* and *Pseudomonas aeruginosa*.

At the concentration used, PAB was effective in overcoming the inhibitory effect of sulfanilamide but not that of PA for *Pseudomonas aeruginosa* (Table 2). In fact, PAB appeared to enhance the antimicrobial activity of PA against *Pseudomonas aeruginosa*. These results can be explained, at least in part, by the observation that PAB itself was inhibitory under these study conditions (Table 2).

TABLE 2. Minimal Inhibitory Concentrations (MIC) of Phosphanilic Acid and Sulfanilamide in the Absence and Presence of Para-Aminobenzoate (PAB) and MIC of PAB alone for *Pseudomonas aeruginosa* and *Escherichia coli* in Basal Salts Supplemented with 20 mM Glucose (BSG)

Agent	MIC (μg/ml)[a] *Pseudomonas aeruginosa*[b]	*Escherichia coli*[c]
Sulfanilamide	200 (600)	200
Sulfanilamide + 200 μg/ml PAB	> 1,000[d]	400
Phosphanilate	20 (100)	< 100[e]
Phosphanilate + 200 μg/ml PAB	< 10[f] (30)	> 1,000[d]
PAB	500 (500)	200

[a] The tube serial dilution technique was used in which the concentration of the agents was increased by increments of 100 μg/ml from 100 to 1,000 μg/ml or by increments of μg/ml from 10 to 100 μg/ml.
[b] The nonparenthetical numbers were results afer 24 h incubation while the parenthetical numbers were results after 48 h incubation.
[c] *Escherichia coli* grew slowly on BSG and turbidity did not occur until in excess of 24 h incubation. Thus, the results shown here represent 48-72 h incubation.
[d] Concentrations > 1,000 μg/ml were not used.
[e] Concentrations < 100 μg/ml were not used.
[f] Concentrations < 10 μg/ml were not used.

Effect of PA on Dihydropteroate Synthase. PA inhibited the synthesis of dihydropteroate by dihydropteroate synthase in extracts of both *Pseudomonas aeruginosa* and *Escherichia coli* (Table 1). As a control, sulfadiazine, which is a well-documented inhibitor of dihydropteroate synthesis, was used. Thus, at a mol:mol ratio of 10:1 of sulfadiazine:PAB, sulfadiazine completely inhibited the synthesis of dihydropteroate by extracts of both *Pseudomonas aeruginosa* and *Escherichia coli*. PA, on the other hand, was a weak inhibitor of dihydropteroate synthase. At a mol:mol ratio of 100:1 with PAB, PA inhibited dihydropteroate synthase activity of *Pseudomonas aeruginosa* and *Escherichia coli* only 22% and 28%, respectively. A mol:mol ratio with PAB of 1,000:1 was required to achieve a high level of inhibition.

DISCUSSION

At the intact cell level, our data showed that Pseudomonas aeruginosa was more susceptible to the inhibitory effect of PA than Escherichia coli, thus confirming observations by others (3), that PAB prevented inhibition by PA of Escherichia coli in BSG medium but not of Pseudomonas aeruginosa and that PAB itself exhibited an inhibitory effect.

At the enzyme level, our results confirmed that PA inhibits dihydropteroate synthase by acting as a competitor of PAB. Thus, PA exerted its inhibitory effect in the same manner as the sulfonamides with which PA is structurally similar. PA, however, was a weak inhibitor of dihydropteroate synthase. Curiously, however, patterns of the susceptibility and resistance of bacteria to PA did not always parallel those of the sulfonamides (unpublished observations).

It is interesting that the dihydropteroate synthases of Pseudomonas aeruginosa and Escherichia coli were about equally affected by PA (actually, the dihydropteroate synthase of Escherichia coli appeared to be somewhat more sensitive to PA than that of Pseudomonas aeruginosa) whereas intact cells of Pseudomonas aeruginosa were much more susceptible to PA than intact cells of Escherichia coli. A probable explanation is that intact cells of Pseudomonas aeruginosa were much more permeable to PA than intact cells of Escherichia coli. Thus, since Pseudomonas aeruginosa can utilize a variety of aromatic substances such as benzoate, catechol, or toluate that are structurally related to PA, it is likely that PA is taken into the cells of Pseudomonas aeruginosa by diffusion and permeation mechanisms physiologically designed for the uptake of aromatic compounds.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG1842	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CI	304
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code)
(U) Role of Thyroid Hormones in Burn Pathophysiology

12. SUBJECT AREAS
06 01 Biochemistry 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
79 08	99 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

a. DATE EFFECTIVE APPROVED BY

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.2	82
89	1.2	86

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: VAUGHAN, G M
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-5416
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Adults; (U) Lab Animals: (U) Rats; (U) Hamsters; (U) RAII

23. (U) To assess alterations in the thyroid axis in burn injury to improve survival in injured soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) To characterize normal nocturnal changes in thyroid hormones in a murine model prior to further evaluation of the effects of injury.

25. (U) 8710 - 8809. Certain aspects of normal thyroid axis function must be better understood before optimal designs can be constructed to assess alterations in this axis after injury. Measurements of hormone dynamics during the dark pahse of the lighting cycle have been helpful in elucidating the functions of other neuroendocrine axes. Therefore, we studied Sprague-Dawley (SD) and Fischer-344 (F) rats in different photoperiodic conditions. Around-the-clock radioimmunoassay measurements of serum thyroxine (T4), triiodothyronine (T3), their free indices (FT4I, FT3I), and thyrotrophin (TSH) were subjected to a statistical program we designed specially to assess the timing of nocturnal hormonal surges without bias due to other rhythmic parameters. Symmetrical lengthening of the dark phase engendered a nocturnal surge of T4 in SD rats. In F rats, nocturnal surges in total and free indices of both thyronines occurred even in a scotoperiod < 12 h, and dark phase lengthening changed the surge timing to indicate entrainment control by lights-on rather than lights-off. Surprisingly, TSH measurements suggested mediation of thyronine changes by a nonpituitary mechanism in both strains. Alteration in assay techniques, leading to assessment of another source of these fluctuations (tissue stores), was begun.

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USGPO 1986-491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: Nocturnal Changes of Thyroid Hormones in a Murine Model Without Burn Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

George M. Vaughan, MD, Lieutenant Colonel, MC
Mary K. Vaughan, PhD*
Russel J. Reiter, PhD*

*Department of Cellular and Structural Biology
The University of Texas Health Science Center
San Antonio, Texas 78284-7877

ABSTRACT

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PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

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Data on photic control of rhythmic thyroid function are few. We exposed male Sprague-Dawley and Fischer-344 rats to 24-h cycles with either 10 h dark (2000-0600 h) or 14 h dark (1800-0800 h) from 21-77 days of age. Groups of 7-9 rats were sacrificed at 2000, 2200, 0100, 0400, 0600, 0800, and 1300 h for assays of serum tetra- and triiodothyronine (T_4, T_3), T_3 uptake (T_3U), free indices (FT_4I, FTT_3I, = T_4, T_3 X T_3U), and thyrotrophin (TSH). In Sprague-Dawley rats in both photic regimens, 24-h rhythmicity of T_4, FT_4I, and TSH was evident, these variables being higher at 1300 h than early in the night. A within-dark-phase rise in T_3 was detected in both conditions, as well as one for T_4 in the long dark condition. Nocturnal changes in this strain were not pronounced enough to detect differences in timing of these fluxes between photic conditions. However, in Fischer-344 rats, nocturnal changes were more pronounced. Also in this strain in both photic regimens, T_4, FT_4I, T_3, and TSH were higher at 1300 h than early in the dark phase. Further, 12-h periodic regressions of data from 2000-0600 h revealed nocturnal fluctuations of T_4, FT_4I, T_3, and FT_3I in both regimens with an amplitude of 10% (T_4, FT_4I) or 19% (T_3, FT_3I) of the overall mean. Acrophases (curve peak times) in 10-h dark were between 0200 and 0300 h. In 14-h dark, acrophases of T_4, FT_4I, T_3, and FT_3I were each delayed (compared to 10-h dark) by about 2 h. Nocturnal fluctuation in TSH was not detected. Despite advanced lights-off, longer darkness delayed T_4 and T_3 flux by 0200 h (similar to the delay of lights-on) in Fischer-344 rats. Lights-on is the photic cue ultimately entraining nocturnal rise of serum T_4 and T_3. Whether this rise is mediated by fluctuations in bioactivity of secreted TSH and/or in iodothyronine disposal is not yet known.

NOCTURNAL CHANGES OF THYROID HORMNES IN A MURINE MODEL WITHOUT BURN INJURY

Burn injury produces changes in many neuroendocrine systems (1-12), including the pituitary-thyroid axis. These changes could represent interaction of the injury with other factors and influences that normally exert control over these systems. A major external influence on several pituitary-dependent functions is the relative lengths and timing of the light and dark periods of the 24-h cycle (13-25). Such photoperiodic influences have been most pronounced in ungulate and hamster species in which marked changes of the reproductive system occur (18-24). Short photoperiod or blinding also lowers thyroid function in Syrian hamsters measured generally at one time during the day (19). The effects of altered photoperiod on patterns of thyroid variables measured around-the-clock are less well studied. However, in rats there are day/night rhythmic fluctuations in serum thyronine concentrations in the standard long laboratory photoperiod (26). Those data also contained preliminary evidence that there were fluctuations in some thyroid variables occurring during the dark phase (scotoperiod) of the cycle. Therefore, we adapted two strains of rats to each of two photoperiodic conditions and sampled serum for assessment of nocturnal fluctuations in thyroid axis hormone concentrations at intervals during both the longer and shorter dark periods.

MATERIALS AND METHODS

Male Sprague-Dawley (SD) and Fischer-344 (F) rats were obtained at 21 days of age. Half the rats of each strain were placed in a light/dark cycle of 14 h light and 10 h dark (LD 14:10) and the other half in LD 10:14. For LD 14:10, lights-off was at 2000 h and lights-on at 0600 h. For LD 10:14, lights-off was at 1800 h and lights-on at 0800 h (a symmetrically 4-h longer dark phase). In both conditions, middark was at 0100 h, and midlight at 1300 h. Ambient temperature was 22°C and food and water were available *ad libitum*. Rats were housed two per clear plastic cage with fragmented corncob bedding. Fluorescent lighting ("cool white") provided an average 60 foot-candle intensity at the front of the cages during the light phase. There was no light during the dark phase.

After 8 wk in either lighting condition, at 77 days of age, the animals were sacrificed for blood and tissue samples by guillotine decapitation. Figure 1 shows the schedule for taking these samples in relation to the two lighting regimes. Groups of rats were sacrificed at 2000 h (just before lights-off in LD 14:10), 2200 h, 0100 h, 0400 h, 0600 h (just before lights-on in LD 14:10), and at 0800 h (just before lights-on in LD 10:14). A group in LD 10:14 was sacrificed

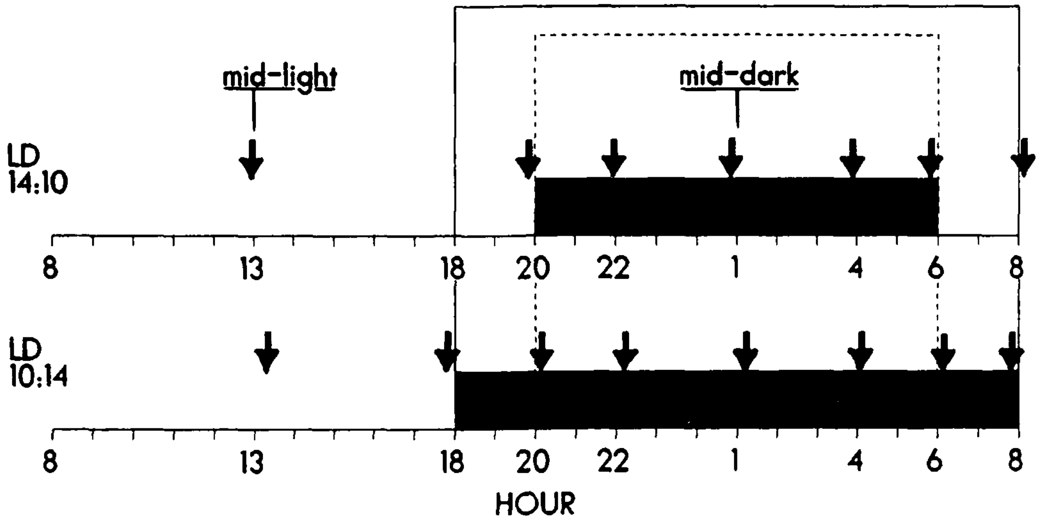

FIGURE 1. Young adult male rats of two strains (SD and F) were housed for 8 wk in either of the two photoperiodic conditions shown (LD 14:10 or 10:14). They were sacrificed (under dim right light if during darkness) by guillotine in groups of 7-9 at the times indicated by arrows. The horizontal bar indicates the length of darkness. The groups that were pinealectomized and maintained in LD 10:14 were sampled at 1300 and 0100 h.

just before lights-off at 1800 h. A group was sacrificed at 1300 h in both lighting conditions. Additional groups that had been pinealectomized (Px) for 8 wk were sacrificed at 0100 h and at 1300 h in the long dark (LD 10:14) regimen. Sacrifice during the dark phase was accomplished under red photographic safe lights (two 25-watt incandescent lamps with Kodak 1A filters) without entry of any other light into the animal room during the dark phase. This dim red light exposure was previously found to have no effect on nocturnal melatonin synthesis. Body weights were recorded prior to sacrifice and trunk serum was saved at -60°C for assays. Within a given strain and lighting condition, group sizes at the various times of sacrifice were 7 to 9 rats, except for pinealectomized groups of 5 rats each. Reproductive organs were dissected and weighed from representative animals under both lighting conditions.

Thyroxine (T_4), triiodothyronine (T_3), and T_3 charcoal uptake (T_3U) (with kits from Diagnostic Products, Los Angeles CA), and thyrotrophin (TSH) (with supplies from NIDADDK, Bethesda MD; sampling volume 200 μl serum) were measured by radioimmunoassay (radioassay for T_3U). Free thyronine indices (FT4I = $T_4 \times T_3U$; $FT_3I = T_3 \times T_3U$) were calculated with use of

the T_3U to correct for changes in circulating thyronine binding. For the TSH assay, the least detectable value was 0.5 ng/ml in buffer, and in separate studies exogenous T_4 administration to rats lowered serum TSH in a dose-related fashion.

Statistical Analyses. Statistical tests were made using the P1D, P7D, and P1R programs of the BMDP package (University of California at Los Angeles) on a VAX 11/780 (27). Between-group statistical comparisons were made by t tests with correction for inequalities of variance and with the Bonferroni correction where applicable for multiplicity of nonindependent comparisons. Hormonal variables within a lighting condition were analyzed for rhythmicity by cosinor regression of a variable, with time expressed as both the sine and cosine of the angle from midnight, on the basis of either 24-h periodicity (24 h = 360°) or 12-h periodicity (12 h = 360°). Overall comparisons between lighting conditions were made by two-way ANOVA, adjusting for variation with time of sacrifice at the time points common to both conditions. Comparisons of timing (acrophase or time of best-fit curve peak) of fluctuation between lighting conditions without interference by parameters of amplitude or mesor were made if the periodic fluctuation was significant ($P < 0.05$) in both lighting conditions. With use of the cosine and sine coefficients and their standard errors (SE) from a 12-h P1R regression (for data in the interval 2000-0600 h) in each condition, a SE elipse was computed for 36 points about the acrophase point for the respective condition in order to describe only the central acrophase angle and the variation in this angle up to 1 SE on either side. This variation (one SE in one condition taken from the acrophase in the shortest angular direction toward the acrophase of the other condition) and the similarly taken acrophase and SE of the other condition were used in t tests (with the residual degrees of freedom from the regressions that computed the angular coefficients and their SE) to compare the nocturnal acrophases between the two lighting conditions. Such tests for acrophase difference between photoperiodic conditions were also made on 24-h and 12-h cosinor regressions of the overall data if such a rhythm was significant in both conditions.

RESULTS

Figure 2 shows body and organ weights. In both strains of rats, the longer dark condition was associated with a slightly but significantly lower body and pituitary weight than that in the shorter dark condition, with a reversal due to Px seen only in the F strain. In the F strain, testis (combined left and right) and prostate weights were lower in the longer dark condition in non-Px animals. Consideration of the organ weights corrected for body weight suggests that some of the organ changes noted above were likely explained by the general

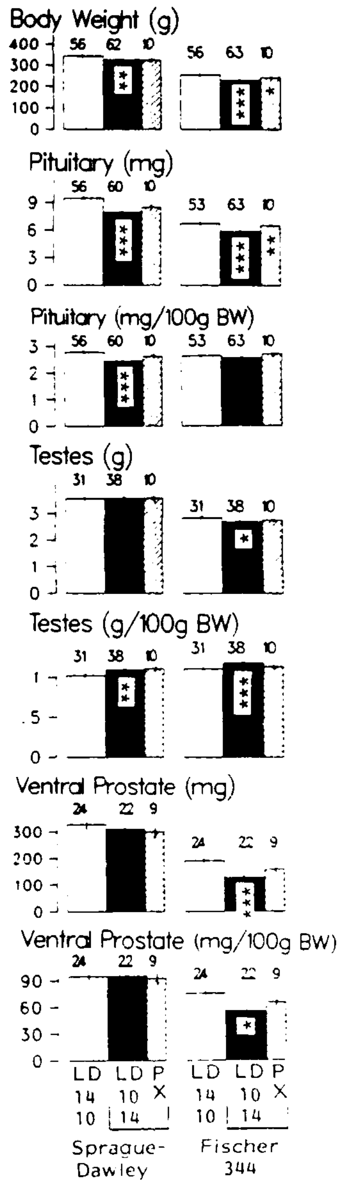

FIGURE 2. Gravimetric data (mean ± SE) obtained on portions of the animals in each condition (LD 14:10, 10:14). Px, pinealectomized at the beginning of the 10:14 condition. The number of animals for which these data are available is above each bar. *P < 0.05, **P < 0.01, ***P < 0.001, LD 10:14 vs. 14:10 or Px vs. the others (intact) in 10:14.

influence reflected in body weight. Exceptions were the long dark phase-related reductions in pituitary weight in SD and in prostate weight in F, both statistically significant after correction for body weight.

Table 1 shows that the 24-h mean was not different between lighting condition for any of the hormonal variables in either strain. Twenty-four-hour rhythmicity was detected (amplitude different from 0) for all these variables in either condition in both strains, except for T_3 in the short dark condition and FT_3I in either condition in SD, and FT_3I in the short dark condition in F. Differences in rhythm timing (acrophase with 24-h data) between conditions were not apparent. Separate analyses of the nocturnal data detected nocturnal fluctuation (rises) in SD rats for T_3 and T_3U (both conditions) and T_4 (long dark condition), and in F rats for T_4, T_3, T_3U, FT_4I, and FT_3I in both conditions. In F rats, the nocturnal acrophase timing for T_4, T_3, FT_4I, and FT_3I was significantly later (by 1.5-3 h) in the longer dark condition. Figure 3 depicts the variation described above. The difference in timing of the nocturnal fluctuations in the F rats for T_4 and FT_4I is reflected by lower group values at middark in the long (vs. short) dark condition. Pinealectomy (Px) did not produce a detectable alteration form the values recorded in the same (long dark) condition without Px at either middark or midlight.

Fluctuations of TSH could be related to those of thyronines only in terms of the general 24-h variation. In both strains and photic conditions, thyronines and TSH appeared higher during the daytime (the midlight point) than at early dark points, reflected in significant daytime 24-h-based acrophases (Table 1). However, the nocturnal fluctuation in thyronines was not associated with statistically detectable similar changes in TSH.

Figure 4 is a polar depiction of the timing of the nocturnal fluctuations of thyroid axis hormones in F rats in which the longer dark condition produced a delay in the fluctuation of T_4, T_3, and their free indices.

DISCUSSION

Syrian hamsters exposed to the long dark condition (LD 10:14 for 2 mon) exhibit profound suppression of the reproductive system reflected in prepubertal testes and prostate size, a response almost totally prevented by pinealectomy (18). Similarly, depression of serum T_4 (usually measured during the day) also occurs in hamsters exposed to 14 h dark per cycle and is prevented by Px (19). In that species, serum testosterone and T_4 are also low after burn injury, responses not prevented by Px (5). In the rats of this study, the changes in body and organ weights in the long dark (compared with values in short dark) were very mild. In F rats, the reduction in prostate weight exceeded that seen in body weight, but it was not significantly reversed by pinealectomy. In either strain, T_4 and T_3 were not different between lighting regimes at 1300 h or in terms of the overall around-the-clock magnitude after accounting for variation with

TABLE 1. Time-Related Fluctuation Parameters of Serum Thyroid-Related Variables

Strain	hor-mone	LD	24-h mean	24-h periodicity: Amplitude	24-h periodicity: Acrophase	24-h periodicity: Acrophase difference	12-h periodicity: Amplitude	12-h periodicity: Acrophase	12-h periodicity: Acrophase difference	12-h periodicity data 20.00-06.00 h: Amplitude	12-h periodicity data 20.00-06.00 h: Acrophase	12-h periodicity data 20.00-06.00 h: Acrophase difference
Sprague-Dawley	T4	14:10	5.45	0.23*	12.98		0.25*	01.92		-	-	
		10:14	5.46	0.35**	12.47	-00.51	0.37***	03.24	01.32	0.34**	03.89	-
	T3	14:10	92.9	-	-		9.00*	02.34		12.38*	02.35	
		10:14	92.1	7.27*	08.66	-	10.63*	03.87	01.52	11.44*	03.78	01.43
	T3U	14:10	46.4	1.77***	17.49		-	-		1.69**	21.50	
		10:14	46.6	1.89***	17.13	-00.36	1.54**	23.49	-	1.45*	22.19	00.69
	FT4I	14:10	2.53	0.17*	15.02		0.14*	01.00		-	-	
		10:14	2.55	0.23**	14.13	-00.89	0.17**	02.29	01.29	-	-	-
	FT3I	14:10	43.2	-	-		4.17*	01.88		-	-	
		10:14	43.1	-	-	-	4.25*	03.35	01.47	-	-	-
	TSH	14:10	3.57	0.83*	08.84		-	-		-	-	
		10:14	3.03	1.14**	12.75	03.91	0.73*	02.11	-	-	-	-
Fischer 344	T4	14:10	5.75	0.47***	10.09		0.56***	02.10		0.70***	02.50	
		10:14	5.70	0.77***	12.49	02.41	0.63***	03.68	01.58*	0.63***	04.54	02.04***
	T3	14:10	99.0	12.62**	08.60		10.30*	02.01		18.80***	02.29	
		10:14	102	20.10***	10.03	01.44	19.74**	03.90	01.89	22.89***	03.79	01.50*
	T3U	14:10	47.1	2.00***	19.17		1.54**	00.04		1.54*	22.79	
		10:14	48.1	1.90**	18.96	-00.21	-	-	-	1.77*	21.35	-01.44
	FT4I	14:10	2.71	16*	12.04		0.32***	01.64		0.31***	02.01	
		10:14	2.73	0.40***	13.71	01.67	0.26**	03.43	01.78*	0.22*	05.04	03.03**
	FT3I	14:10	46.8	-	-		5.92**	01.61		8.78**	01.97	
		10:14	48.3	8.91***	11.15	-	7.57*	03.68	02.07	7.93**	03.81	01.84*
	TSH	14:10	1.61	0.53***	10.36		-	-		-	-	
		10:14	1.78	0.73***	11.95	01.59	-	-	-	-	-	-

From regressions of the 24-h data separately for 24-h and 12-h periodicity and of data only from 2000 to 0600 h for 12-h periodicity. The 24-h mean and amplitude are in units for the respective variable (see Fig 3), and the acrophase (time of nocturnal cosinor peak) and its difference between photoperiodic conditions are in decimal hours. There are no significant differences in 24-h mean (after accounting for variation with time) between LD 14:10 and 10:14. *P < 0.05, **P < 0.01, ***P < 0.001 for amplitudes vs zero amplitude; for acrophase difference, LD 10:14 vs. 14:10. Entries are absent if rhythms were not significant.

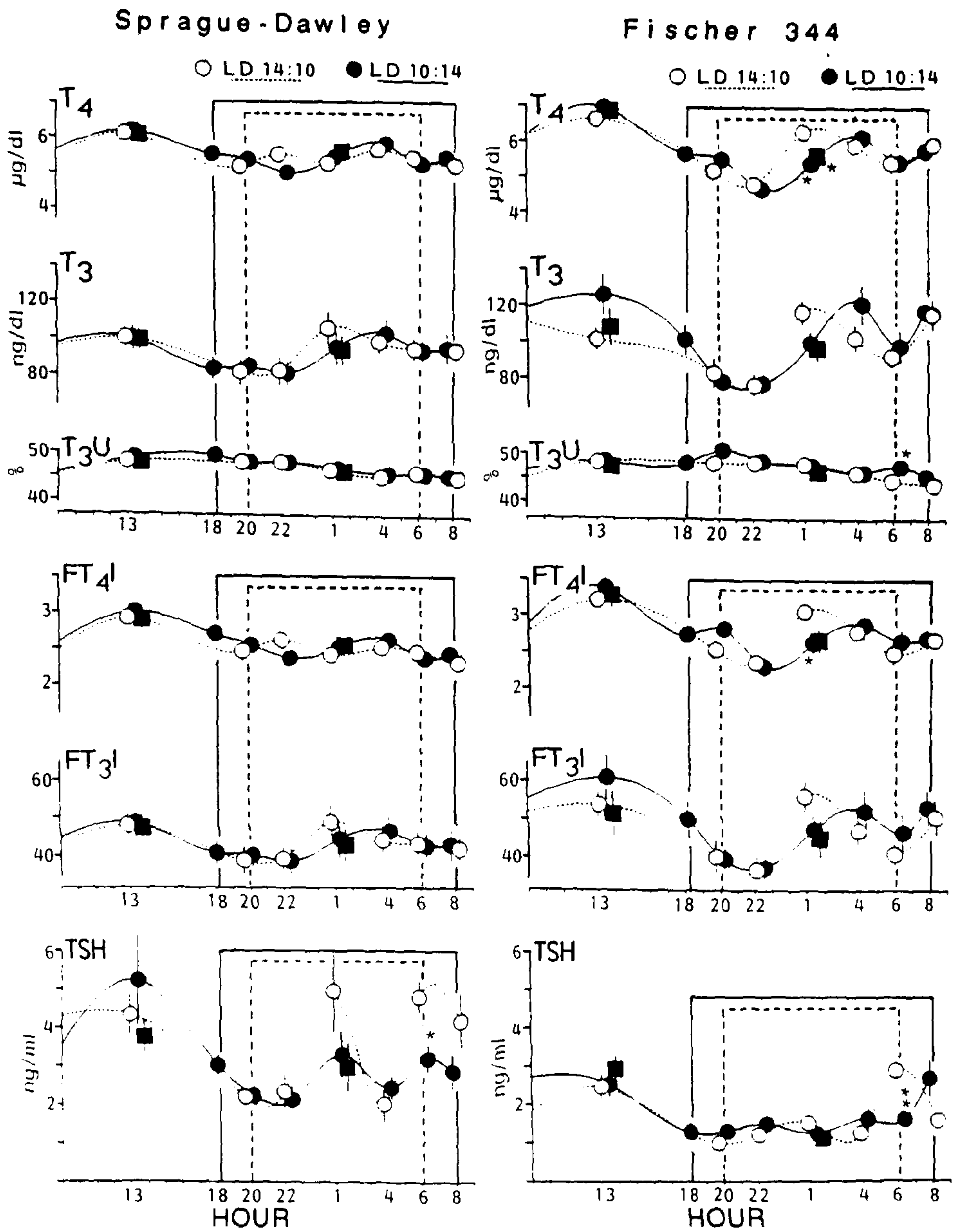

FIGURE 3. Serum thyroid-related variables (mean ± SE) in each time group. The vertical lines indicate the lengths of the dark phase for LD 14:10 (dashed) and LD 10:14 (continuous). FT4I and FT3I, free thyronine indices (T4 or T3 X T3U). TSH by NIH antibody, least detectable 0.5 ng/ml. The closed squares (1300 and 0100 h) represent pinealectomized rats in LD 10:14. *P < 0.05, **P < 0.01 vs. LD 14:10 at the same time point. The curved lines represent spline fits to the means.

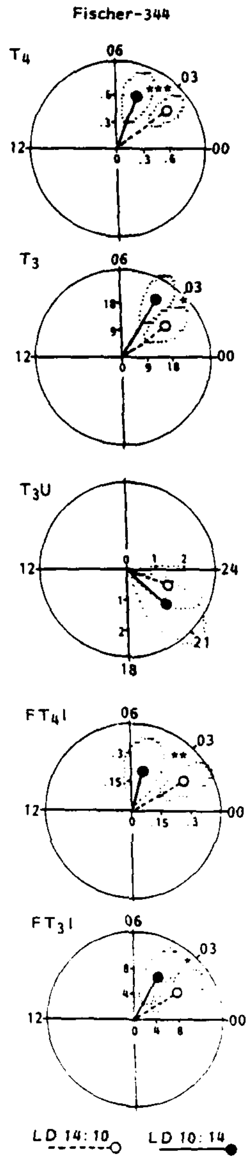

FIGURE 4. Polar representation of the nocturnal amplitude and acrophase (with 95% confidence elipses) for serum thyroid variables. Data only from 2000-0600 h (12-h periodic regression) were used, because separate nighttime peaks are often seen in thyroid hormones. The radial distance from the origin is the amplitude and the angle is the acrophase (time of the nocturnal peak). Numbers on the circle represent hours from midnight (00 or 24). TSH from both strains and all hormones from SD are not shown because of absence of rhythmicity or absence of an acrophase difference between LD 14:10 and 10:14. *P < 0.05, **P < 0.01, ***P < 0.001, LD 14:10 vs. 10:14 (with respect only to acrophase).

time (ANOVA). Pinealectomized groups in the long dark condition had values indistinguishable from those of the intact animals in the long dark condition. Though this is consistent with no effect of Px, pinealectomized animals were sampled at only two time points. Testing of the effect of the pineal on the rhythmic pattern of thyronines would require sampling of pinealectomized groups at all time points.

Serum T_4 and T_3 (and their respective free indices) showed fluctuation during the night, seen best in F rats. In these rats, the amplitude of the nocturnal rise was about 10% of the overall mean for T_4 and FT_4I and about 19% for T_3 and FT_3I. In SD rats, the short dark condition was associated with an apparently similar nocturnal rise in T_3 and FT_3I in the graphically presented data, and this was statistically significant for T_3. In this strain under the long dark condition, a nocturnal rise in T_4 also became significant, suggesting that longer darkness may facilitate expression of an oscillator controlling nocturnal fluctuation in serum T_4 in SD rats. However, in this strain, the nocturnal rises were not pronounced enough to detect a difference in their timing between the two lighting regimens.

In contrast, in the F rats, nocturnal rises in iodothyronines were statistically significant in both lighting regimens, and a significant delay in their timing was detected in the longer dark condition. For T_4 and FT_4I, this was reflected by a difference between group values at the 0100 h time point (lower in longer dark, $P < 0.05$) and also by a significant interaction term ($P < 0.05$) in the ANOVA testing the influences of nocturnal sampling time (2000-0600 h) and of lighting condition. In either condition, the serum iodothyronine peak tended to occur about 4 h before lights-on. Interestingly, this meant that even though the long dark condition included a relatively phase-advanced (2-h earlier) time of lights-off with respect to that in the short dark condition, the serum iodothyronine pattern in long dark became phase-delayed by the same magnitude (2 h) of the relative phase delay in the time of lights-on. This response is compatible with an oscillator (as part of the endogenous clock) controlling serum iodothyronine levels, which oscillator can be entrained preferentially by the cue of lights-on. Such entrainment over time would allow anticipation of the time of lights-on by a rise in circulating iodothyronines.

The messenger controlling these fluxes in iodothyronine concentrations is open to speculation. The lack of detection of a nocturnal fluctuation in TSH probably is reliable in terms of the assay, because the fluctuation between 1300 h and early night is evident in the graphic display and is reflected in a significant 24-h cosinor analysis in both strains in both conditions. This could explain similar day-to-early-night variation in thyronines that is also evident. Thus, the lack

of a significant within-night TSH change fitting the pattern of the thyronines might be explainable by much more evanescent rises in TSH, partly missed by the sampling schedule. An impression of this possibility is obtained from the apparently somewhat erratic TSH curves in SD rats (Fig 3), though nocturnal fluctuation was not substantiated statistically. In the F rat curves, wherein the nocturnal thyronine changes were even more definitive, there is apparently even less nocturnal variation in TSH. Further, in short dark, the end-dark-phase TSH rise is advanced compared to that in the long dark regimen, suggesting that the nocturnal surge in thyronine concentrations suppressed TSH and release from this suppression was earlier in the short dark condition in which the iodothyronines fell sooner.

This suggests the possibility that peripheral disposal (perhaps including deiodination of T_4 and T_3) slows during the hormone surge and accounts for it. This would mean that the endogenous oscillator sends a message to deiodinating tissues such as liver and kidney. One tissue recently found to be active in T_4-to-T_3 deiodination, brown adipose tissue (BAT), is controlled by the sympathetic nervous system (28,29). The sympathetic nervous system stimulates a rise in activity (melatonin synthesis) of another organ, the pineal, at night (7). Because serum T_4 concentrations are so much higher than those of T_3, a rise in BAT activity would probably have more of an effect (if any) in raising serum T_3 than lowering T_4. If disposal of T_4 by *alternative* routes in *other* tissues is simultaneously inhibited, both serum T_4 and T_3 might rise. BAT T_4 deiodination is a critical step in sympathetic-mediated cold-induced thermogenesis (29). It is not yet known if deiodinase is involved in burn-induced thermogenesis. It will be important to investigate the time-linked patterns of serum thyronines, the activity of deiodinases in several tissues, and the kinetics of T_4 and T_3 in a burn model.

PRESENTATIONS/PUBLICATIONS

Vaughan GM: Control of TSH after burn injury. Presented at the 4th Annual Meeting of the US Army Regional American College of Physicians, San Franciso, California, 25 October 1987.

Vaughan GM: The pineal and its relation to burn injury and human physiology. Presented to the Anatomy Department, Mahidol University, Bankok, Thailand, 18 July 1988.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CA	305
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) Inequality of VA/Q Ratios Following Smoke Inhalation Injury and the Effect of Angiotensin Analogues

12. SUBJECT AREAS

06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	99 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

APPROVED BY: Basil A. Pruitt Jr

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		a. FISCAL YEARS	c. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	2.0	68
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e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: IKEUCHI, H
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-7832
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): SAKANO, T
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22. KEYWORDS (Precede EACH with Security Classification Code) (U) Inhalation Injury; (U) Cardiac Output; (U) Indicator Dilution; (U) Ventilation-Perfusion Ratio; (U) Cobra Venom;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (U) Lab Animals: (U) Sheep; (U) RAII

23. (U) To evaluate the effect of smoke inhalation on pulmonary ventilation and perfusion. To study the effects of resuscitation fluid volume and qualities on pulmonary ventilation-perfusion ratios and microvasculature. A literature search was performed and indicated no duplication of effort.

24. (U) Ventilation-perfusion ratios will be measured utilizing the six-inert gas technique. These pulmonary variables will be correlated with standard cardiopulmonary variables before and after the induction of inhalation injury and subsequent treatment with different types and volumes of resuscitation fluid. Lung lymph will be collected and a specific gravity method for estimation of extravascular lung water volume will be used to assess the pathophysiologic mechanisms of pulmonary edema formation after smoke inhalation. A platelet-activating factor antagonist (CV-3988) will be used to estimate the role of platelet-activating factors in edema formation following smoke inhalation.

25. (U) 8710 - 8809. Ventilation-perfusion alterations following smoke inhalation injury have been established. The effects of resuscitation have been studied. Modifications of the six-inert gas technique to solve problems of low sensitivity in the gas chromatography-mass spectrometer have been developed. The use of other gases in efforts to solve these problems has also been initiated.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: INEQUALITY OF VA/Q RATIOS FOLLOWING SMOKE INHALATION INJURY AND THE EFFECT OF ANGIOTENSIN ANALOGUES: Role of the Complement System in Smoke Inhalation Injury - Effects of Decomplementation on Cardiopulmonary Function

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Takeshi Shimazu, MD
Hisashi Ikeuchi, MD
Robert C. Allen, MD, PhD
Gene B. Hubbard, DVM, MS
Tsutomu Sakano, MD
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

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PROJECT TITLE: INEQUALITY OF VA/Q RATIOS FOLLOWING SMOKE INHALATION INJURY AND EFFECTS OF ANGIOTENSIN ANALOGUES: Role of the Complement System in Smoke Inhalation Injury - Effects of Decomplementation on Cardiopulmonary Function

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: Takeshi Shimazu, MD
Hisashi Ikeuchi, MD
Robert C. Allen, MD, PhD
Gene B. Hubbard, DVM, MS
Tsutomu Sakano, MD
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

The role of the complement system in the progression of respiratory failure following smoke inhalation injury was studied in animals pretreated with cobra venom factors (CVFs) to effect decomplementation before smoke exposure. Two different CVFs from Naja naja (CVF-n) and Naja haje (CVF-h), with different modes of complement activaton, were used. Twelve sheep were treated with 200 U/kg of either CVF-n or CVF-h. The animals were exposed to moderate smoke inhalation injury 24 h after CVF treatment. Six control sheep received smoke inhalation alone without CVF pretreatment. Cardiopulmonary function and CBC were measured throughout the study until the animals were sacrified for gross and histologic evaluation 24 h after smoke exposure.

Significant hypoxia and pulmonary hypertension were induced by CVF injection (CVF-n > CVF-h). None of the control sheep died by 24 h after exposure, while 1 died in each of the CVF-treated groups. There was no significant difference among the surviving sheep in cardiopulmonary function after smoke inhalation. Necropsy, however, revealed the presence of bacterial colonies in the necrotic tracheal debris in both CVF groups which was not seen in the controls.

In conclusion, this study demonstrated that cardiopulmonary derangement following smoke inhalation injury is not attributable to complement activation alone and that administration of CVF does not alleviate pulmonary damage, but rather appears to compromise the defense mechanism of the lung.

ROLE OF THE COMPLEMENT SYSTEM IN SMOKE INHALATION INJURY - EFFECTS OF DECOMPLEMENTATION ON CARDIOPULMONARY FUNCTION

We have developed a dose-responsive and reproducible sheep model of smoke inhalation injury, and successfully employed this model to examine the physiologic and pathologic changes that follow smoke inhalation (1). In smoke-exposed animals, an inflammatory response, evident as early as 3 h postinjury, increases in severity at 6, 12, and 24 h postinjury. Although arterial PO_2 and lung mechanics are maintained under mechanical ventilation 3 h postinjury, hypoxia and inflammatory changes develop fully by 24 h postinjury. Animals exposed to a CO gas mixture did not develop hypoxia at 3, 24, or 72 h following exposure and no pathologic changes were observed (2,3). These findings suggest that the toxic products in smoke trigger an inflammatory response and that the resulting inflammation is essential to the pathophysiology of respiratory insufficiency. The delayed onset of respiratory insufficiency observed in some burn patients with evidence of smoke inhalation injury may be a consequence of burn-related initial depletion or suppression of immune responsiveness, e.g., neutropenia and low alternative pathway complement capacity, predisposing such patients to the development of pulmonary sepsis.

In this study, we examined the influence of the complement system and PML on the pathophysiology of respiratory insufficiency following smoke inhalation injury. Decomplementation of animals by pretreatment with cobra venom factors (CVF) has been reported to affect various immunologic reactions (4). CVF, per se, is reported to produce lung injury (5,6), but CVF has also been reported to protect the lung from inflammatory injury associated with sepsis (7). We examined the effects of two different types of CVF, one from *Naja haje* (CVF-h) and one from *Naja naja* (CVF-n). The former lacks the ability to generate C5 convertase activity (8); the C5-C9 complements are preserved, and generation of C5a-like peptides, known to be potent stimulants of PML chemotactic activity, should not occur using this CVF. However, CVF-n totally activates complement, and as such, should cause pulmonary accumulation of PMLs (5). Use of both CVF-n and CVF-h may provide information with regard to the importance of C5a-dependent PML accumulation in pulmonary injury and possible clinical application of CVF to prevent smoke inhalation injury.

MATERIALS AND METHODS

Animals. Eighteen male sheep weighing 35.4 ± 4.9 kg were used for this study. The sheep were divided into three groups. Group 1 (n=6) received smoke alone and served as the control group. Group 2 (n=6) was preteated with CVF-n and exposed to smoke 24 h following treatment. Group 3 (n=6) was pretreated

with CVF-h and exposed to smoke 24 h following treatment. Animals were chronically instrumented with peripherial venous and arterial lines and a Swan-Ganz catheter for at least 3 days prior to smoke exposure (Group 1) or CVF pretreatment (Groups 2 and 3). Animals were kept in a cage that allowed free movement and access to food and water and was housed in a temperature and humidity-controlled room.

CVF Pretreatment. Two types of CVF were obtained from Cordis Laboratories, Inc. (Miami FL). The first was CVF from the common Indian cobra (*Naja naja*, CVF-n, Catalog #750-007, Lot #S4005) and the second was from the Egyptian cobra (*Naja haje*, CVF-h, Catalog #750-005, Lot #34025). These two CVF have different modes of complement activation (8) and are separated from the neurotoxin, hemolysin, and lecithinase of the raw venom by column chromatography and/or gel ultrafiltration (9). Lyophilized CVF was reconstituted with 5 ml distilled water for each vial. Two hundred units of CVF per kilogram was infused into each study animal IV over 5 min. Blood pressure (BP) and PAP were recorded continuously from prior to infusion until BP and PAP returned to baseline levels. The animals were observed for 24 h, with cardiopulmonary measurements obtained as detailed below.

Smoke Exposure. Twenty-four hours following CVF pretreatment, the sheep in Groups 2 and 3 were exposed to smoke to produce a moderate degree of smoke inhalation injury as described previously (1). Group 1 was exposed to smoke without CVF pretreatment. The spontaneously breathing animals were observed for 24 h, with measurement of cardiopulmonary indices at scheduled intervals.

Cardiopulmonary Monitoring. Cardiopulmonary indices, including cardiac output, BP, PAP, CVP, PCWP, arterial and mixed-venous blood gas analyses, CBC, and blood chemisties were measured (1). A total of 16 sets of measurements were obtained for each CVF-treated sheep and 8 sets of measurements were obtained for control group sheep according to the study design shown in Table 1.

Pathology. Necropsies were performed on all surviving sheep at the end of the study and on sheep dying spontaneously.

Statistical Analysis. Comparisons among the three groups were made by ANOVA.

RESULTS

Effects of CVF Treatment. Soon after the initiation of CVF infusion, the sheep became restless, with irregular breathing in association with alterations of BP and PAP (Fig 1). Such changes in behavior and BP were more prominent in sheep infused with CVF-n. There was a rapid elevation of PAP with CVF-n,

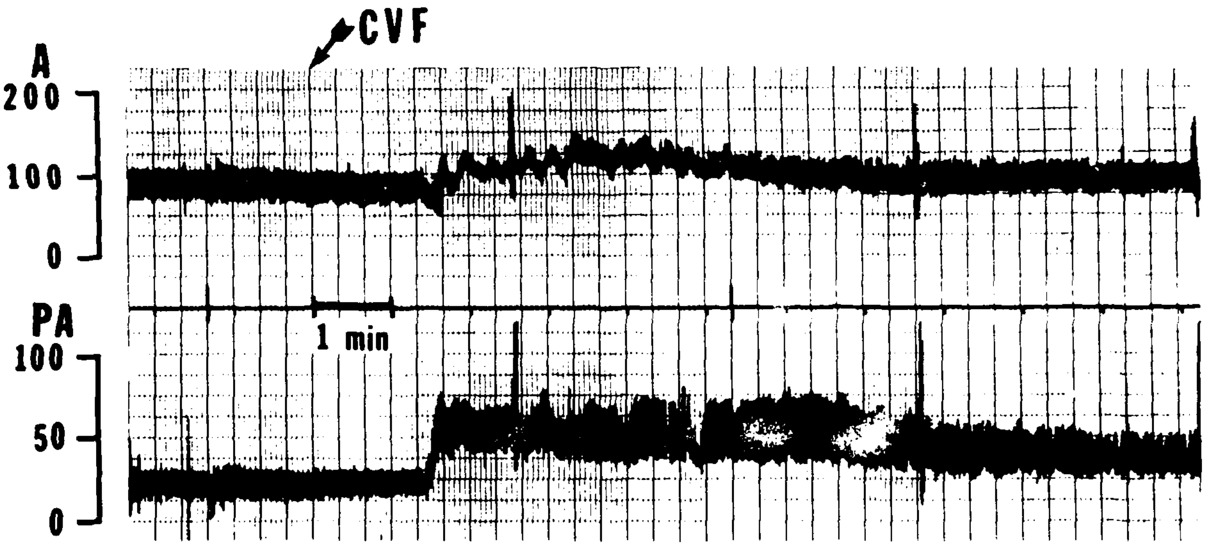
TABLE 1. Experimental Design for Blood Sampling and Cardiopulmonary Measurements

Number*	Time Period	Control Group	Treatment Groups
1	Pretreatment	No	Yes
2	5 min	No	Yes
3	30 min	No	Yes
4	1 h	No	Yes
5	2 h	No	Yes
6	3 h	No	Yes
7	6 h	No	Yes
8	12 h	No	Yes
9	24 h/Presmoke	Yes	Yes
10	30 min	Yes	Yes
11	1 h	Yes	Yes
12	2 h	Yes	Yes
13	3 h	Yes	Yes
14	6 h	Yes	Yes
15	12 h	Yes	Yes
16	24 h	Yes	Yes

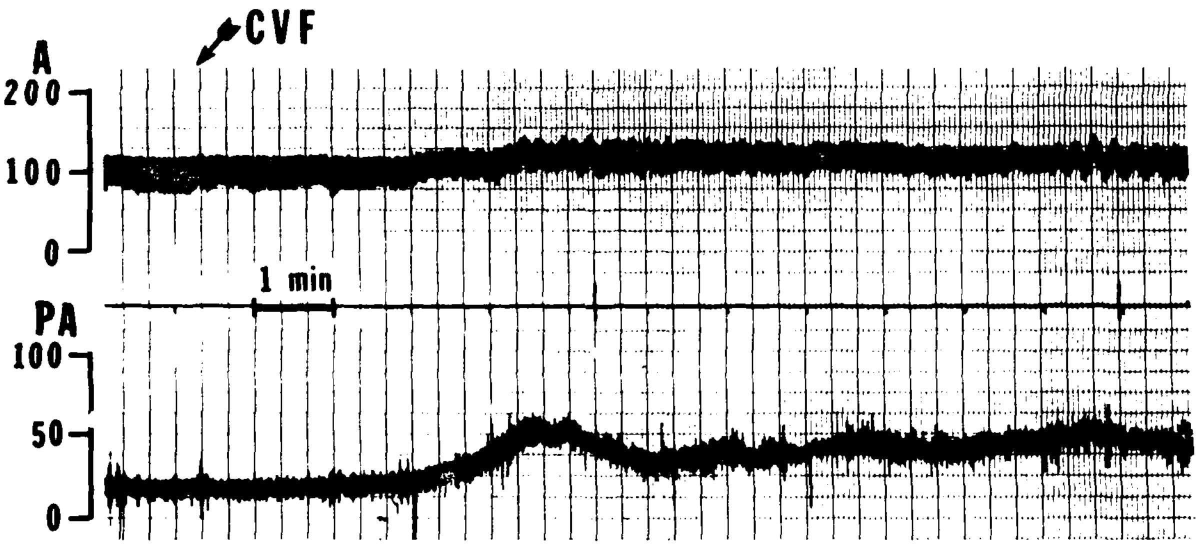
*These numbers correspond to the numbers on the time axis shown in the figures.

from 18.0 ± 1.4 to 52.7 ± 1.2 mmHg and a lag time of 44 ± 3 sec, while CVF-h slowly elevated PAP from 18.5 ± 1.5 to 44.7 ± 2.4 mmHg with a lag time of 178 ± 7 sec (Fig 1). BP and PAP returned to baseline levels within 30 min using either CVF.

CVF infusion also produced a transient decrease in PaO_2, cardiac output, and WBC (Figs 2-4). Hypoxia induced by CVF-n (mean decrease in PaO_2, -15.9 ± 4 mmHg) was greater than that induced by CVF-h (-6.3 ± 1.8 mmHg), being maximum within 30 min and returning to baseline levels by 2 h (Fig 2).



A



B

FIGURE 1. Changes in systemic arterial (A) and pulmonary artery (PA) pressure induced by CVF injection. **A.** CVF-n. **B.** CVF-h. CVF-n induced rapid and significant elevation in PAP and instability in A, while CVF-h caused slow and moderate changes.

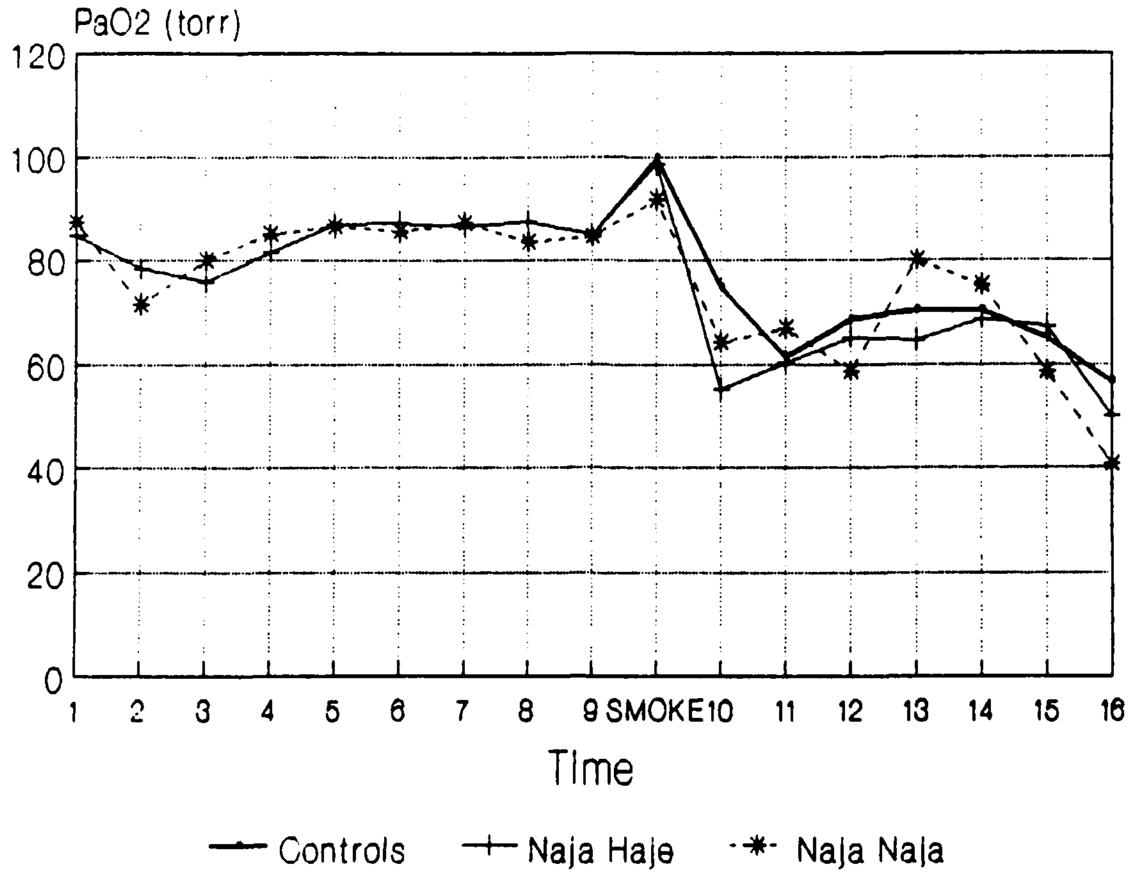


FIGURE 2. PaO_2 changes following CVF treatment and smoke exposure. Mean values are shown. Numbers on the time axis correspond to the numbers listed at Table 1. CVF treatment induced only transient hypoxia (CVF-n > CVF-h). There was no significant difference after smoke exposure among the three groups.

Cardiac output decreased slightly immediately after CVF administration from 5.1 ± 0.2 to 3.3 ± 2.4 l/min by 5 min for Group 2 and from 5.2 ± 0.2 to 4.6 ± 2.1 l/min by 5 min for Group 3 (Fig 3). Cardiac output returned to the normal range by 30 min in both groups.

Both CVFs induced initial leukopenia at 5 min (10600 ± 2320 to 9100 ± 2520/mm^3 for Group 2 and 11300 ± 4410 to 4400 ± 2930/mm^3 for Group 3) followed by leukocytosis at 24 h (Fig 4). These changes are primarily attributable to changes of neutrophils. Neutrophils decreased from 4533 ± 2346 to 411 ± 529/mm^3 at 5 min following CVF-h infusion and from 4290 ± 1130 to 3463 ± 1860/mm^3 following CVF-n infusion. Lymphocytes were not affected significantly by either CVF, but there were two remarkable peaks of eosinophils at 30-60 min and 24 h after CVF

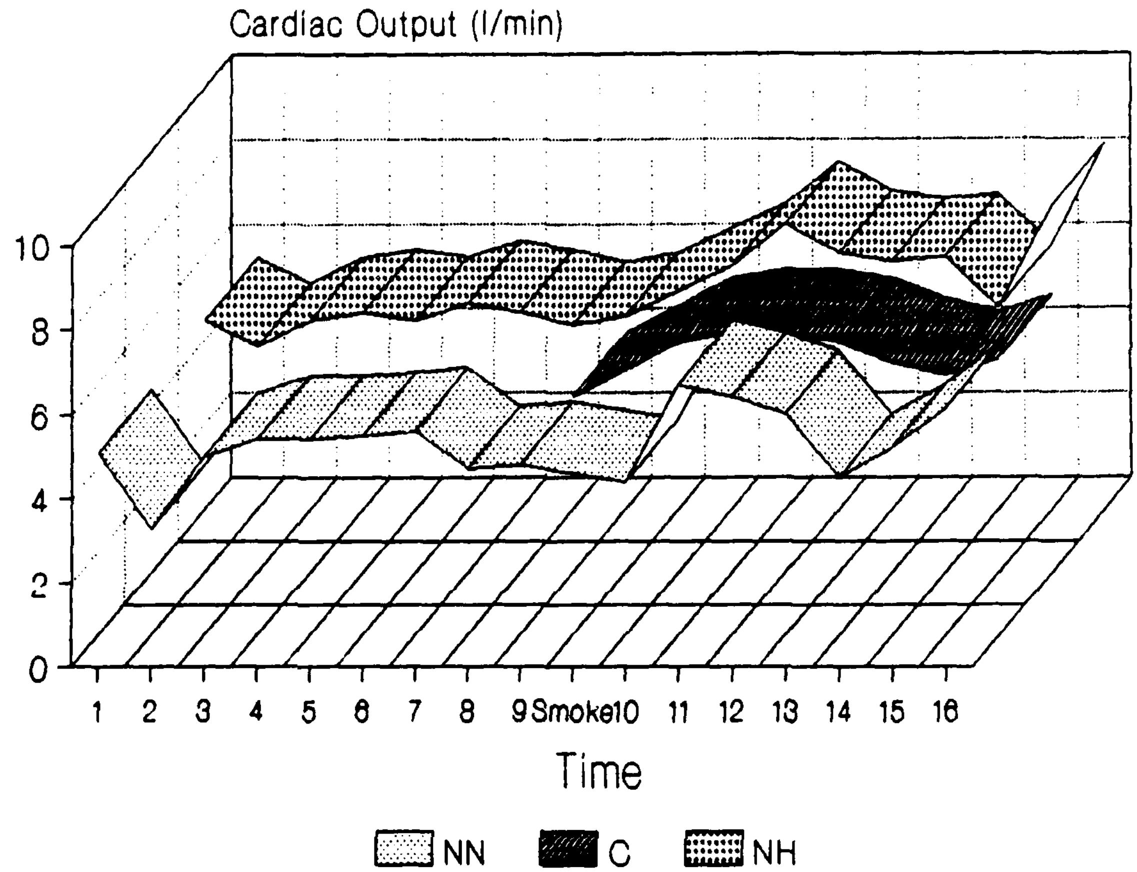


FIGURE 3. Changes in cardiac output. CVF treatment induced a moderate decrease in cardiac output (CVF-n > CVF-h), but cardiac output returned to baseline level by 30 min. Following smoke exposure, cardiac output showed a moderate increase, which was attributable to physiologic exposure (time 13 on the X axis) or thereafter is related to hypoxia from the respiratory injury.

administration. Platelets remained constant throughout the study period. There was no significant effect on blood lactate levels.

Effects of Smoke Exposure. When the CVF-pretreated sheep were exposed to smoke, symptoms and cardiopulmonary indices did not alter from the control sheep. Average peak COHb levels after smoke exposure were 65.9 ± 5.2%, 68.3 ± 8.5%, and 65.2 ± 3.1% for Groups 1, 2, and 3, respectively. All sheep regained spontaneous breathing by 5 min after smoke insufflation.

Changes of PaO_2 and cardiac output induced by smoke exposure were identical for the three groups (Figs 2 and 3).

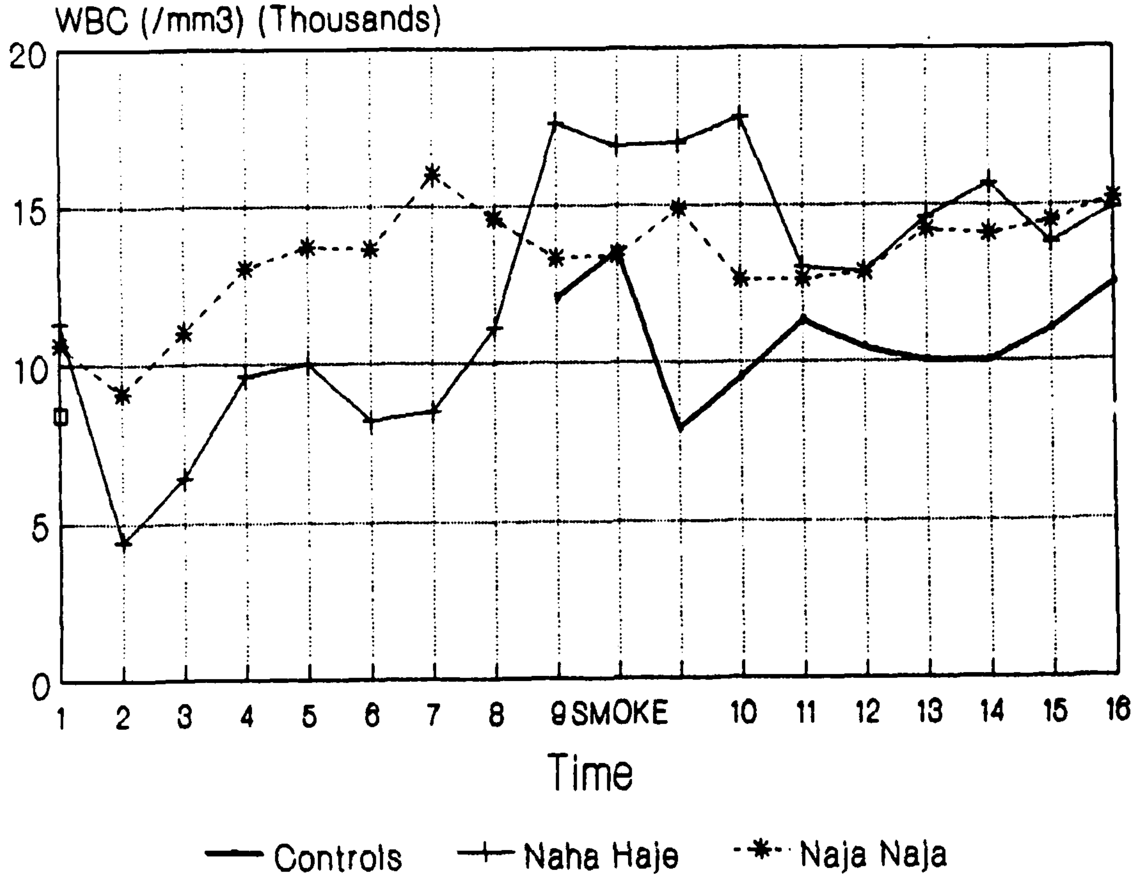


FIGURE 4. Changes in the WBC counts. CVF treatment induced transient leukocytopenia (CVF-n > CVF-h) followed by significant leukocytosis. Smoke exposure caused a decrease in complement system in the activation of WBCs following smoke exposure.

However, one sheep in each of the CVF-treated groups died with significant respiratory distress between 6 and 12 h following smoke exposure. None of the control sheep died during the 24-h study period.

WBCs showed a markedly different response to smoke exposure (Fig 4). For the control group, WBCs increased slightly at 5 min and then decreased significantly at 30 min from 12000 ± 7260 to 7900 ± 2690/mm^3. The CVF-treated groups did not show such a decrease. At 24 h, however, there was no significant difference in WBC, neutrophil, or lymphocyte counts among the three groups.

Although no significant differences in cardiopulmonary function were observed in surviving animals, histologic

FIGURE 5. *Photo of a sheep trachea 24 h after moderate smoke exposure and 48 h after receiving 200 U/kg CVF-n that illustrates severe necrosis of respiratory epithelium (E) and a marked inflammatory cell infiltration (I). Note the large bacterial colonies (B) on the surface and the apparent inability of the inflammatory cells to control this growth. This bacterial colonization is not seen in similar smoke exposed sheep at 24 h. (H&E X 500).*

examination revealed a striking difference in the CVF-treated animals. Colonization of bacteria on the tracheal surface was observed in CVF-treated animals (Fig 5), while control animals had no bacterial colonies.

DISCUSSION

Bauman (8) has reported different modes of complement activation *in vitro* by CVF-n and CVF-h (8). CVF-h lacks the ability to generate C5 convertase activity, and as such, the C5-C9 complements are preserved, while CVF-n totally activates complement (Fig 6). Most animal studies employing CVFs have not taken into count the differnces of the two CVFs. The differences in CVF activites of complement may influence the injury to various organs (5); but after the complement system

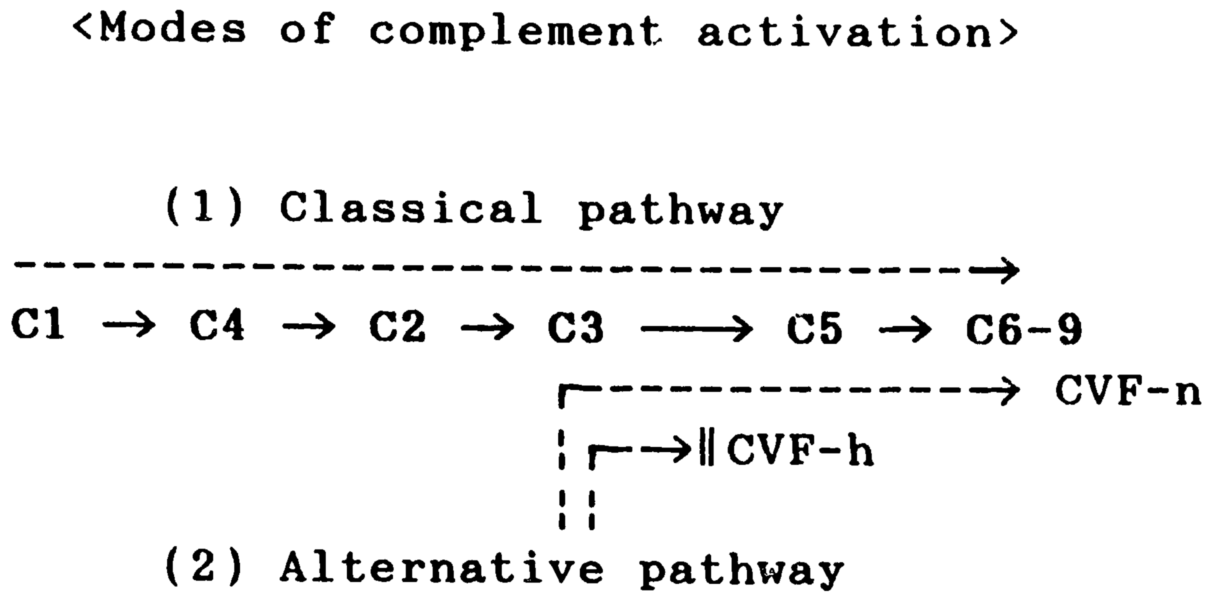


FIGURE 6. Modes of complement activation by CVF-n and CVF-h. Both CVFs activate the alternative complement pathway via C3. CVF-h only partially activates the complements since it lacks the ability to generate C5 convertase activity, while CVF-n activates the complements totally.

has been depleted, the animal may because resistance to another stress that is mediated via the complement system (7).

Although Bauman reported the in vitro difference between the two CVFs, their effect on cardiopulmonary function has not been directly compared and this is the first report that demonstrates a difference of the two CVFs in vivo. As expected from its inability to generate C5a, CVF-h had less effect on systemic BP, PAP, cardiac output, and PaO_2 (Figs 1-3). However, CVF-h caused greater leukocytopenia followed by significant leukocytosis (Fig 4). These changes and differences cannot be attributed to contaminants in the CVF products, such as endotoxin or phospholipase A (lecithinase), since the products were separated from neurotoxin, hemolysin, and lecithinase and each lot was tested for lecithinase activity (9). To exclude the endotoxin effect, we tested each injection fluid for endotoxin using an endotoxin-specific analysis (10,11). Endotoxin levels in the infusates ranged from 2.6 (CVF-n) to 26.3 (CVF-h) ng/ml, corresponding to 7.5 to 76.3 EU/ml (EU = endotoxin unit of USP reference standard endotoxin, *Escherichia coli* 0113). Despite such differences, CVF-n and CVF-h showed consistent effects on cardiopulmonary indices that appear to be related to endotoxin concentration. Futhermore, in a few animals, we added polymyxin B to the infusate to neutralize endotoxin and the response was identical

to that without polymyxin B (12,13), suggesting that endotoxin was not responsible for the observed responses. On the other hand, their effects on cardiopulmonary function were only transient and complement activation alone does not cause persistent lung dysfunction (ARDS), circulatory derangment, or pulmonary hypertension.

The analysis of sheep sera for complement component levels (CH50, C3a, C5a) has not been completed due to technical difficulties related to the animal species (sheep). Consequently, the degree of the decomplementation remains unknown in these study animals. However, the dose employed (200 U/kg) should have been enough to completely activate the complement system, since 20 U/100 g of CVF injected IV into guinea pigs causes a 95% reduction in hemolytic C3 levels by 3-4 h after injection (9). Other studies using CVFs from the same manufacturer (Cordis Laboratories, Inc.) with doses < 200 U/kg have demonstrated complement depletion in various species, including pigs, rabbits, mice, and rats (8,14-16).

It has been postulated that pulmonary injury associated with burns in general and smoke inhalation injury in particular is related to complement activation and subsequent leukocyte margination in the lung. Therefore, our initial hypothesis was that decomplementation might reduce lung injury from smoke inhalation injury and that CVFs, particularly CVF-h, might be therapeutically applicable. The initial effects of CVF on cardiopulmonary function were only transient and complement activation alone did not cause persistent circulatory changes or lung dysfunction. Moreover, following inhalation injury, neither CVF treatment brought about improvement in cardiopulmonary function. On the contrary, it resulted in increased mortality and increased susceptibility to infection.

In conclusion, we have demonstrated the different effects of the two CVFs on cardiopulmonary function. This study also suggests that complement activation alone does not induce persistent lung dysfunction. The results further indicate that immunomodulation by decomplementation with CVF pretreatment does not alleviate lung injury associated with smoke inhalation injury and may well impair host defenses.

PRESENTATIONS/PUBLICATIONS

Shimazu T: Effects of smoke inhalation injury on ventilation-perfusion ratio of the lung. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

Shimazu T: Carbon monoxide elimination process in acute and chronic CO poisoning: Comparison by two-compartment

analysis. Presented at the 20th Annual Meeting of the American Burn Association, Seattle, Washington, 25 March 1988.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA311499	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	IA	306
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model

12. SUBJECT AREAS
06 01 Biochemistry 06 13 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

APPROVED BY Basil A. Pruitt

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1.8	58
c. TYPE	d. AMOUNT	89	1.8	61
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR SHIPPEE, R L
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-7138
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Thermal Injury; (U) Zinc Homeostasis; (U) Immunocompetence; (U) Lab Animals: (U) Rats; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To determine the changes in zinc metabolism caused by burn and infection in a murine model as manifested by altered mechanisms at the whole body, organ, and molecular levels. Information obtained from these studies will be related to the role of these changes in septic complications and be used to determine optimal levels of zinc supplementation in burned humans. A literature search was performed and indicated no duplication of effort.

24. (U) Studies will be conducted to determine the mechanisms involved in the phenomena seen in previous studies. These studies involve the isolation of T lymphocytes and macrophages with subsequent in vitro culturing of these cells from the different burn and zinc treatment groups. It is hypothesized, based on data from the literature concerning zinc deficiency, that the T lymphocytes from the zinc-deficient, burned rats will show less proliferation when stimulated with mitogen. It is further hypothesized that the proliferation will be increased when these cells are co-cultured with the macrophages from the zinc-supplemented, nonburned rats.

25. (U) 8710 - 8809. Previous studies with this model have shown that no significant increases in endogenous fecal and urine excretion were detected during the first 10 days postburn. Burn injury and burn injury plus zinc deficiency caused a decrease in total peripheral blood T cells. However, burned/zinc-deficient animals showed an increase in T suppressor cell populations. The results from the plaque assay have shown a supression in antibody response in burned/zinc-deficient animals. These studies have demonstrated a modulating effect of zinc nutriture on various aspects of the immunological system after burn injury.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Ronald L. Shippee, PhD, Captain, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

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PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: Ronald L. Shippee, PhD, Captain, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Clinical and animal studies suggest that burn injury induces alterations in zinc metabolism. It has been well established that zinc nutriture can cause alterations in the immune response. However, there is a paucity of information concerning the interrelationship between burn injury, zinc nutriture, and the immune response. In the present study, rats were subjected to a full-thickness dorsal scald injury of 30% of the total body surface and then maintained on sufficient or deficient zinc intake. The rats were immunized with sheep red blood cells on postburn day 6. Ten days postburn, the rats were sacrificed, spleens excised, and spleen lymphocytes isolated and used in a Jerne plaque assay to determine the number of plaque-forming cells (PFCs). The burn/zinc-sufficient regimen significantly increased the PFC response when compared to control nonburned zinc-sufficient or zinc-deficient rats. Burned rats that were maintained on a zinc-deficient regimen showed a significant decrease ($P < 0.05$) in PFCs when compared to burned rats maintained on a zinc-sufficient regimen. This study indicates an interaction of zinc in the primary humoral immune response during recovery from a thermal injury.

PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL

A considerable body of evidence exists relating zinc nutriture to immunocompetence in both animal research and clinical studies (1-3). Zinc deficiency produces abnormalities in thymus morphology which have been shown to interfere with both T cell dependent and T cell independent maturation (4-5). Indications of abnormal development of thymus tissue suggest perturbations in T lymphocyte function which are essential for cell-mediated immune response.

In view of the well documented immunosuppression often seen in burned humans and laboratory animals, we investigated the possible interactions of zinc metabolism, immunocompetence, and burn injury. In preliminary investigations, we have used flow cytometry techniques to quantify changes in T lymphocyte subset populations after a 30% total body surface area burn injury in zinc-supplemented and zinc-deficient rats. The data revealed a significant change in the T lymphocyte subset population (6). It was found that rats that were burn-injured and zinc-restricted for 10 days postburn had a significant increase in T suppressor cells. This report describes the results of studies designed to determine the effect of zinc nutriture on the immunocompetence in the burn rat model using a functional assay of the immune system, the Jerne plaque assay.

MATERIALS AND METHODS

Experimental Animals. A total of 72 male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Houston, TX) weighing $\pm$ 350 g were used for these studies. Rats were housed in individual stainless steel cages, given distilled deionized water _ad libitum_, and maintained on a 12:12 light:dark cycle. The rats were fed a semi-purified diet (Ziegler, Inc., PO Box 95, Gardners, PA) designed to meet all the nutrient requirements of the adult rat except for zinc. All rats were fed this zinc-deficient diet (< 0.5 ppm) _ad libitum_ for 2 wk and given daily subcutaneous injections of zinc sulfate (1 mg/kg). This modality of zinc administration was used for a number of reasons. In our previous work, we studied the effect of burn injury on fecal endogenous zinc excretion. To quantitate this important route of zinc excretion, a low zinc diet was fed along with subcutaneous administration of zinc as zinc sulfate. Also, this method of zinc administration simulates the zinc given in TPN solution, a feeding regimen often used in severely burned patients.

After a 2-wk equilibration period, the rats were weighed and assigned to one of the following regimens in a manner that equalized mean body weight among the treatment groups (n=6):

Burn Sufficient (BS) = 30% TBSA Burn, 1 mg Zn/kg

Burn Deficient (BD) = 30% TBSA Burn, Saline

Control Sufficient (CS) = No Burn, 1 mg Zn/kg

Control Deficient (CD) = No Burn, Saline

All rats were anesthetized with sodium pentobarbital (35 mg/kg IP) prior to burn injury. The dorsal area was shaved and exposed to a 90°C water bath for 10 sec to produce a 30% full-thickness burn injury. Six days postburn, all rats were immunized with sheep red blood cells (SRBCs). A 20% solution of SRBC in a volume of 0.2 cc was injected into a tail vein. Four days after immunization with SRBCs, the rats were sacrificed. Preliminary studies had shown that the peak response occurred on postburn day 4 for both control and burned rats. This design was replicated 3X for a total of 24 rats per treatment group.

Hemolytic Plaque Assay. Spleens were placed in a disposable Petri dish and teased apart with stainless steel spatulas in 1 ml HBSS. Cells were aspirated from the Petri dish into 15-ml tubes and washed twice with HBSS (centrifuge washing was done at 150 g for 10 min). Cells were pipetted onto 4-ml Ficoll-Hypaque gradient and centrifuged at 450 g for 30 min. Suspended cells were counted in a Coulter Counter (Coulter Electronics, LTD, Model Zm, Northwell Drive Luten, Beds, England) and equalized to a concentration of 1 X 10^6 cells/ml. The following solutions were then pipetted into a 5-ml test tube placed in a 42°C water bath: 0.050 ml SRBCs (14% in minimum essential medium (MEM)) and 0.400 ml agarose-MEM (1% agarose and 2 X MEM at a 1:2 ratio). The tubes were removed from the water bath and 0.100 ml of the suspended cell solution pipetted into the tube. This mixture was spread onto a precoated 75 X 50 mm glass slide with a stirring rod and allowed to solidify at ambient temperature for 2 min. The slides were incubated in MEM for 1 h at 37°C in 5% CO_2. After incubation, the slides were rinsed with saline and reincubated with fresh MEM-guinea pig complement mixture (1:30). After 45 min of incubation, the slides were rinsed in saline and fixed in acetone and ethanol. Splenic plaque-forming cells (PFCs) were quantified using a dissecting microscope.

Tissue and Plasma Zinc Analysis. Zinc concentrations in liver tissue were determined by perfusing the liver with physiological saline, then removing approximately 700 mg of liver tissue that was dried at 80°C for 12 h in a vacuum oven, after which 10 ml of acid solution containing 21.5% perchloric acid, 7.0% sulfuric acid, and 71.5% nitric acid was added. After concentrating on a hot plate to approximately 2 ml, the wet-washed tissue was diluted to 25 ml with distilled deionized water and aspirated directly into the atomic absorption

spectrophotometer (Perkin-Elmer, Model 5000 AAS, Norwalk CT) for determination of zinc concentrations.

To 1 ml of plasma was added 1 ml of a 20% trichloroacetic acid solution. This solution was vortexed and then centrifuged for 20 min at 500 g. Zinc concentrations of the supernatant were determined by atomic absorption spectrophotometry.

Statistical Analysis. A computer software program (Statistical Analysis System, SAS Institute, Inc., Version 5.0, 1985, Cary, NC) was used to obtain descriptive statistics and perform a one-way ANOVA. When the F statistic was found to be significant ($P < 0.05$), a Duncan's multiple range test (SAS User's Guide, Statistics, 1982, SAS Institute, Inc.) was used to test for differences among the treatment means.

RESULTS

The three consecutive repetitions of the study design and the mean PFCs per experimental group for each repetition are shown in Table 1. The total PFC response for all three studies is shown in Figure 1. The data in Figure 1 were normalized by dividing all values within each repetition by the mean value for the zinc-sufficient group of that repetition. The Duncan's multiple range test showed that the PFC response of the BS group was significantly increased above other treatment groups ($P < 0.05$).

The weight changes of the rats from the burn day until day of sacrifice are shown in Table 2. The CS group gained a mean of +13 g over the 10-day period as opposed to +10, +3, and +2 g in the CD, BD, and BS groups, respectively.

The zinc-deficient animals showed a significantly ($P < 0.05$) lower plasma zinc concentration compared to the supplemented groups (Table 2). Zinc supplementation caused a significant ($P < 0.05$) increase in hepatic zinc concentration in the burn-injured rats.

DISCUSSION

A large amount of data exists concerning alterations in cell-mediated responses following burn injury. Increased suppressor cell activity has been described in a number of clinical studies involving burn and other trauma injuries (7-10). Considerably less attention has been given to alterations in humoral responses in burn patients. Considering that bacteremia in thermally injured patients is a persistent problem which can contribute to a significant increase in mortality, studies involving changes in humoral immunity appear relevant (11).

TABLE 1. PFC Response (PFCs/1 X 10^6 Lymphocytes)

	n	BS	BD	CS	CD
Repetition 1	24	63	46	28	25
Repetition 2	24	100	66	43	39
Repetition 3	24	53	30	27	17

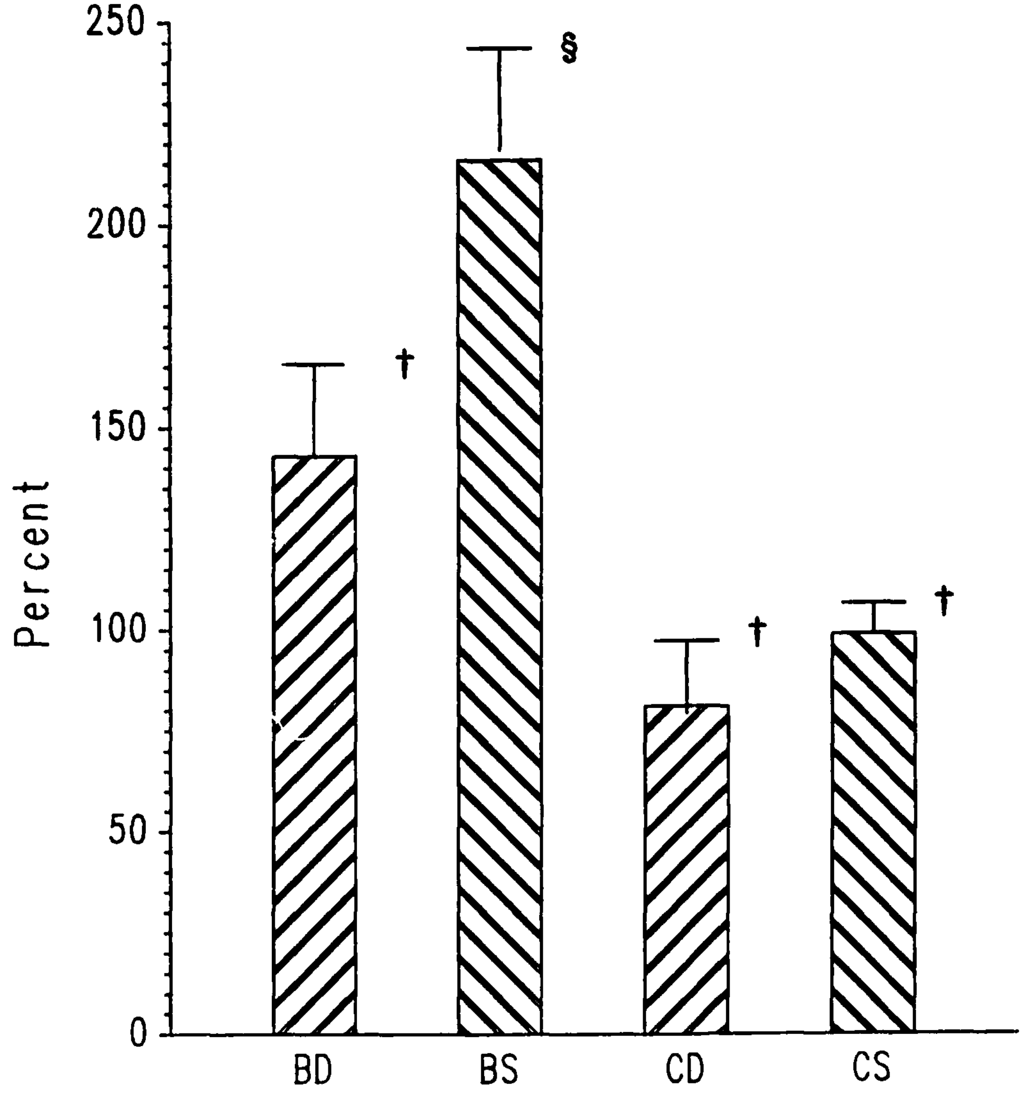

FIGURE 1. Primary humoral response. Treatments sharing common symbols are not significantly different (P < 0.05).

TABLE 2. Mean Body Weight, Plasma, and Hepatic Zinc*

Treatment Groups	n	Starting Body Weight (g)	Ending Body Weight (g)	Body Weight Change (g)	Plasma Zinc (μg/dl)	Hepatic Zinc (μg/dl)
BS	18	341	344	$+3^{a}$	137^{b}	121^{b}
BD	18	342	340	-2^{b}	72^{b}	86^{b}
CS	18	341	354	$+13^{b}$	149^{b}	76^{b}
CD	18	342	352	$+10^{b}$	75^{a}	94^{a}

*Means within variables sharing common superscripts are not significantly different ($P < 0.05$).

Alexander and Moncrief (12) reported that the humoral response to an antigenic stimulus following thermal injury was found to have a better than normal response after a booster injection of tetanus toxoid, but lower than normal after injection of heterologous erythrocytes. Rats, after a 30% full-thickness burn, showed suppression of the primary and anamnestic responses to human erythrocytes (12). In contrast, no deficit was seen in the primary immune response to *Pseudomonas aeruginosa* or to somatic typhoid antigen.

More recent research has shown that lymphocytes isolated from peripheral blood of burn-injured patients with 25-85% total body surface area burns produced significantly greater quantities of IgG and IgM in nonmitogen-stimulated cultures when compared to controls. Cultures from the burned patients failed to increase production of antibodies when stimulated with PWM (13). In studies of antibody production in burned patients, hemagglutination and RIA showed persistent depression in the humoral response (14).

Soderberg *et al* (15) reported the effect of burn injury on the secondary response to SRBC in a murine model. The results of the Jerne hemolytic plaque assay showed that burn injury caused an increase in PFC response in lymphocytes isolated from spleen tissue.

The controversy that exists concerning the effect of humoral response in burned patients and laboratory animals can be due to various reasons. Results of various assays designed to assess immunocompetence can be influenced by a number of variables, i.e., type of antigen, route of antigen administration, and dose of antigen. An additional variable, and one that is often not well defined in most studies, is the nutritional state of the subjects. It is well documented that zinc nutriture can have profound effects on immunocompetence. In the present study, the rats maintained on adequate zinc intake showed an elevated PFC response when compared to the nonburned control rats.

The increased primary response of the burned zinc-sufficient rats is in contrast to findings by Alexander and Moncrief (12) using a similar animal model. One possible explanation could be the time of antigen administration. Our primary immunization was administered on postburn day 6 with the response assayed on postburn day 10, while Alexander and Moncrief reported results only out to postburn day 4. An additional explanation may be the assays used to assess the primary response. Antibody titer was used by Alexander and Moncrief while our research involved a PFC assay. The PFC assay measures not only the ability of antibody to recognize specific cell surface antigens, but also the ability of the antibody to bind complement leading to lysis of the SRBC.

To assure that the increased primary response was not due to a spontaneous polyclonal expansion due to burn injury, we used spleen lymphocytes from SRBC-immunized rats in a PFC assay containing either SRBC or human red blood cells (HRBC) as the target cells (Table 3). The SRBC target cells gave the predicted response while the HRBC target cells elicited no PFC response.

TABLE 3. SRBC/HRBC Cross-Reactivity in Burned/Zinc-Sufficient Rats (n=8)

Immunization Antigen	Assay Antigen	PFCs/1 X 10^6 Lymphocytes
Saline	SRBC	No Response
Saline	HRBC	No Response
SRBC	HRBC	No Response
SRBC	SRBC	37

Zinc restriction after burn injury caused a significant ($P < 0.05$) reduction in the primary response to a T cell-dependent antigen when compared to burned animals maintained on adequate zinc intake. Although, the mean PFC response for the nonburn control rats maintained on restricted zinc was lower than the control, zinc-sufficient rats, the means were not found to be significantly different. We conclude that these results indicate an interrelationship between zinc nutriture, burn injury, and the ability of the rats to mount a primary immune response.

The specific nature of the zinc effects on immunocompetence is unclear. One mechanism may involve the zinc-dependent thymic hormone which is known to induce intrathymic and extrathymic T cell differentiation (16). The effect of zinc on thymic involution has been reported using antithymulin monoclonal antibodies together with immunofluorescence assays (17). The results indicate that the thymulin molecule binds zinc within the thymus and induces a conformational change necessary for its biological activity. It has been argued that short-term dietary intervention need not directly affect peripheral T cell functional capacity (18). Using a cross-over type design, these researchers have shown that when macrophages from mice fed adequate zinc were co-cultured with T lymphocytes from zinc-restricted mice, cell proliferation was returned to normal.

Future studies will investigate the interaction between zinc nutriture, burn injury, and immunocompetence. These studies will be designed to delineate the mechanism(s) involved and how the perturbations seen in immunocompetence and mortality are related.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA313320	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	IA	307
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Evaluation of Serum Visceral Protein Levels as Indicators of Nitrogen Balance in Thermally Injured Patients

12. SUBJECT AREAS

06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 10	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

a. DATE EFFECTIVE APPROVED BY [signature]

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.5	30
89	0.1	1

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR CARLSON, D
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-6532
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (if available) CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nitrogen Balance; (U) Serum Visceral Protein; (U) Albumin; (U) Transferrin; (U) Retinol-Binding Protein;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Volunteers: (U) Adults; (U) RAII.

23. (U) The purpose of this study is to assess whether circulating levels of albumin, prealbumin, transferrin, or retinol-binding protein can be used as indicators of nitrogen balance in burned soldiers. A literature was performed and indicated no duplication of effort.

24. (U) Baseline serum visceral protein levels will be measured on postburn day 5 following stabilization of the patient's fluid status. Serum levels will be repeated every 3 days until postburn day 30. Changes from baseline level will be correlated with nitrogen balance.

25. (U) 8710 - 8809. This project was approved as minimal risk protocol by the US Army Institute of Surgical Research Human Use Committee on 11 September 1987. Data collection has been completed in 10 patients enrolled in the study.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * USGPO 1986-491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Evaluation of Serum Visceral Protein Levels as Indicators of Nitrogen Balance in Thermally Injured Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Dawn E. Carlson, RD, Major, SP
William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Evaluation of Serum Visceral Protein Levels as Indicators of Nitrogen Balance in Thermally Injured Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: Dawn E. Carlson, RD, Major, SP
William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

We measured serum albumin, prealbumin, transferrin, and retinol-binding protein and determined nitrogen balance in 10 thermally injured adult patients. Nitrogen balances were compared with serum visceral protein concentrations to determine whether one or more of these proteins could be used clinically as an indicator of nitrogen balance. Although small, positive correlations were noted, no useful predictive relationship could be found between visceral protein concentration and nitrogen balance. These data indicate that serum visceral protein concentrations are not clinically useful predictors of nitrogen balance in patients with burns.

EVALUATION OF SERUM VISCERAL PROTEIN LEVELS AS INDICATORS OF NITROGEN BALANCE IN THERMALLY INJURED PATIENTS

The importance of providing adequate calories and protein for healing of serious burn injury is well recognized. What methodology to use in monitoring the adequacy of intake remains a problem. Nitrogen balance is a generally accepted method of determining the efficacy of a nutritional regimen. However, accurate determination of intake and output of nitrogen is labor-intensive, and therefore, impractical for general clinical use.

Measurement of serum visceral protein levels has been suggested as a way to assess the efficacy of intravenous nutrition in general surgical patients. Church and Hill (1) measured serum albumin, prealbumin, transferrin, and retinol-binding protein and found that changes in prealbumin levels correlated with change in nitrogen balance in 54 general surgical patients receiving intravenous nutrition at least 2 wk. Whether prealbumin or one of the other visceral proteins may also be a good indicator of nitrogen balance in patients with thermal injury was not known.

To use serum protein levels as nutritional monitors, one must first recognize deviations which occur in these measurements secondary to thermal injury. Serum albumin levels are decreased following burn injury due to increased loss of albumin into the burn wound, although albumin synthesis rates are increased (2). Additionally, serum albumin levels may be affected by treatment measures or complications which may occur in patients with thermal injury. For example, exogenous infusion of albumin may artificially raise the serum level. Serum transferrin is decreased following burn injury and blood loss or blood transfusion may further alter the measurement (3). Decreases in prealbumin and retinol-binding protein have been described (4). Serum IgG levels are lowest within 48 h following thermal injury and subsequently rise to normal or supranormal levels over a 2-4 wk period (5,6).

This study proposed to ascertain whether measurement of serum albumin, prealbumin, transferrin, or retinol-binding protein could be useful as an indicator of nitrogen balance in patients with thermal injuries.

MATERIALS AND METHODS

Number of Patients. Nitrogen balance and visceral protein levels were measured in 10 patients admitted to the US Army Institute of Surgical Research with thermal injuries.

Inclusion Criteria. Patients meeting all of the following criteria were eligible for enrollment in this study:

1. Patients hospitalized for burn injuries comprising > 20% of the total body surface area.

2. Male or female patients > 18 yr of age. Female patients were previously surgically sterilized, were postmenopausal (> 45 yr of age and the lack of menstrual periods for > 1 yr), or had a negative pregnancy test.

Exclusion Criteria. Patients meeting any of the following criteria are excluded from participation in this study:

1. Outpatients.

2. Patients with contact electrical or chemical burns.

3. Patients who are pregnant.

Study Design. An assessment of nutritional status was performed for each patient upon entry into the study. This assessment included preburn height/weight, history of any recent weight changes, history of any drug/alcohol abuse, evaluation of the patient's preburn nutrition, and history of any medical diseases.

Nitrogen balance was measured every third day. Intake data was collected for the 24-h period corresponding to the 24-h urine collection. Total calorie, protein, fat, and carbohydrate intakes were calculated. All intravenous, enteral, and oral intake was recorded. Information for oral diet consumption was obtained by weighing quantities of foods served to and returned by the patients.

Twenty-four hour urine was collected and aliquots are analyzed for total nitrogen content. Nitrogen loss across the burn wound was estimated using the formula of Waxman _et al_ (7) as follows:

$$\text{g nitrogen/24 h} = 0.1 \times \text{TBSA} \times \%\ \text{TBSA Burn} \times 0.8$$

where TBSA = total body surface area. A corrective factor, as recommended by Waxman _et al_, was used for the effect of silver sulfadiazine application on protein loss across burn wounds. An assessment of the percentage of open burn wound was made each data collection week for use in the above calculation. A factor of 4 g was used to estimate fecal and other integumental losses of nitrogen. The patient was weighed on each data collection day.

Statistical Analysis. The direction of the change in nitrogen balance was compared to the direction of change for the visceral protein levels using Chi-square and linear regression analyses.

RESULTS

Ten patients (9 males, 1 female) were enrolled in this study. The age of these patients ranged from 20-62 yr and burn size from 20-69.5% of the total body surface area.

Nitrogen balances were compared with each of the four serum proteins measured to determine whether one or more of these visceral proteins could be used clinically as an indicator of nitrogen balance. Small, positive correlations were noted, but no useful predictive relationship could be found between visceral protein concentrations and nitrogen balance.

DISCUSSION

We felt the long half-life of serum albumin might limit its usefulness in monitoring day-to-day changes in nitrogen balance but chose to measure it since serum albumin levels are frequently used as nutritional markers and are included in prognostic indices. The lack of correlation between serum albumin levels and nitrogen balance was not surprising. A similar result was found by Starker et al (8) who evaluated serum albumun and body weight as indicators of nitrogen balance during nutritional depletion and repletion in 19 patients repleted with TPN. Likewise, no change in serum albumin levels was reported by McCauley and Brennan (9) who measured these levels in 139 cancer patients receiving TPN.

With the shorter half-lives of transferrin, prealbumin, and retinol-binding protein, we felt it more likely that one of these three protein concentrations would correlate with nitrogen balance changes. Smale et al (10) reported serum transferrin concentration changes correlated with nitrogen balance changes during starvation, oral repletion, and parenteral repletion in a study performed with primates. Tuten et al (11) demonstrated significantly increased levels of preablumin in the presence of positive nitrogen balance in 16 patients on parenteral or entereal nutrition and suggested use of prealbumin concentration as an early indicator of anabolism. Fletcher et al (12) compared transferrin and prealbuminous indicators of nitrogen balance in 16 patients requiring TPN or enteral nutrition support and recommended serum transferrin levels as a clinical indicator.

Unlike these authors, we found no useful correlation between nitrogen balance and any of these serum proteins in our patients with burns. Potentially, the difference may have been due to the multiple blood transfusions our patients received.

Tuten et al (11) excluded patients receiving blood or blood products from participation in their study. Our patients frequently require transfusions and 8 of our 10 patients received blood transfusions during the study period. Tuten et al also eliminated patients whose nutritional therapy was changed or interrupted during the final 3 days of the 7-day study. We included all patients regardless of feeding regimens since our goal was to ascertain whether these parameters would be useful in our usual clinical situation. Smale et al (10) used nonstressed animals, and therefore, had no clinical variables affecting serum protein levels or dietary manipulations. Our results were consistent with those of Shenkin et al (4) who found no difference in serum visceral protein levels in trauma patients receiving vs. not receiving amino acids.

Nitrogen balance is a well recognized method used to assess adequacy of dietary regimens. Obtaining an accurate nitrogen balance determination is difficult. Errors in both intake and output measures are possible. To minimize these errors, food served to and returned by patients were weighed to determine protein intake accurately. Twenty-four hour urine samples were collected by an ICU nurse trained in this procedure and accustomed to collecting data for research protocols. Although these urine samples were analyzed for total nitrogen content, intestinal and burn wound losses were estimated. Also, nitrogen balance does not necessarily equate to nitrogen utilization, particularly with high intake levels typical of patients with burns.

This study demonstrates that serum visceral protein levels are not reliable predictors of nitrogen balance in patients with burns. Further research is needed to determine a method of monitoring the adequacy of nutritional regimens. Possible methods include stable isotope techniques.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA313374	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&B(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	IA	308
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Medium-Chain Triglyceride Utilization in the Thermally Injured Patient

12. SUBJECT AREAS
06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 10	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.3	5
89	0.3	5

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): CARLSON, D
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Thermal Injury; (U) Nutrition; (U) Medium-Chain Triglycerides; (U) Long-Chain Triglycerides; (U) Metabolism;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (U) Volunteers: (U) Adults; (U) RAII

23. (U) The purpose of this study is to examine the ability of thermally injured patients to use medium-chain triglycerides when administered in significant quantity as an effective energy source to maintain nitrogen balance. A literature search was performed and indicated no duplication of effort.

24. (U) Resting energy expenditure will be measured on postburn day 10 and the patient started on enteral nutrition consisting of a carbohydrate load of 5 mg/kg/min with the residual kilocalorie requirements administered as long-chain triglycerides. After a 3-day stabilization period, metabolic measurements will made. Finally, the patient will be changed to a nutrition regimen consisting of a carbohydrate load of 3.8 mg/kg/min and the remaining necessary kilocalories will be administered as 90% medium-chain triglycerides and 10% long-chain triglycerides.

25. (U) 8710 - 8809. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the fourth quarter of fiscal year 1987.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE — U.S.G.P.O. 1986-491-003/30329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Medium-Chain Triglyceride Utilization in the Thermally Injured Patient

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Theresa A. Graves, MD, Captain, MC
Dawn E. Carlson, RD, Major, SP
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Medium-Chain Triglyceride Utilization in the Thermally Injured Patient

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Theresa A. Graves, MD, Captain, MC
Dawn E. Carlson, RD, Major, SP
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

The optimal nutritional support program for the thermally injured patient has yet to be defined. The ability of burn patients to use fat, normally administered as long-chain triglycerides, as an effective energy source has been questioned. Few studies comparing the efficacy of medium-chain triglycerides to standard carbohydrate and long-chain triglycerides regimens have been performed. This study was designed to use medium-chain triglycerides, when administered in significant quantity, as an effective energy source to maintain nitrogen balance. Two patients out of a projected 10 have been enrolled and these patients have completed the study.

MEDIUM-CHAIN TRIGLYCERIDE UTILIZATION IN THE THERMALLY INJURED PATIENT

The optimal nutritional support program for the thermally injured patient has yet to be defined. Various studies have questioned the ability of burn patients to use fat, usually administered as long-chain triglycerides (LCT), as an effective energy source (1-3). Few studies comparing the efficacy of medium-chain triglycerides (MCT) to standard carbohydrate and LCT regimens have been performed. The purpose of this study is to examine the ability of thermally injured patients to use MCT when administered in significant quantity as an effective energy source to maintain nitrogen balance.

Multiple studies have documented that carbohydrate is used preferentially over fat as an energy source when both are administered to injured animals and humans (4-7). However, there is a physiological limit to the amount of carbohydrate which can be effectively utilized by man. Burke et al (8) has suggested that 5 mg/kg/min is the amount of carbohydrate which can be administered before excessive CO production occurs. Excess carbohydrate administered is first transformed to fat, a process which has a RQ of 8, meaning for every molecule of oxygen utilized, 8 molecules of CO are produced. The fat may then be used as an energy source after this conversion. In thermally injured patients, it is usually impossible to meet the increased kilocalorie requirements using carbohydrate alone if one adheres to this limit. Fat administered as LCT can be given to supplement the carbohydrate load and meet energy requirements even though the LCT may be utilized ineffectively.

Recent studies have suggested that MCT may be utilized more effectively than LCT by injured animals (2-3,9). In comparison to LCT, MCT are not stored in liver or fat deposits and are quickly oxidized when administered (10). Maiz et al (3) has demonstrated in a rat burn model that MCT in combination with carbohydrate are just as effective as carbohydrate alone in maintaining nitrogen balance. However, Stein et al (4) has demonstrated that when MCT are adminstered as the sole energy source, there is a negative nitrogen balance, decreased protein synthesis, and decreased peripheral fat stores as compared to carbohydrate alone.

On a cellular level, Goodwin et al (1) has investigated the ability of hepatocyte mitochondria from burned rats to oxidize both MCT and LCT. Oxidation of LCT was decreased when compared to normals while beta oxidation of MCT was increased.

These studies suggest that MCT administered in combination with carbohydrate could serve as an effective energy source in thermally injured patients. The safety of administration of MCT to humans has been documented (10). No untoward effects of

these compounds have been reported to date (11-12). Excessive ketone production does not occur and ketoacidosis has not been a problem. The RQ has been noted to decrease after administration, suggesting utilization as an energy source.

The objective of this study is to determine the effects of MCT on the respiratory quotient and nitrogen balance as compared to LCT.

MATERIALS AND METHODS

Number of Patients. Ten consecutive patients will be enrolled in the study. Two patients have been enrolled to date.

Criteria for Admission into the Study. Patients admitted to the US Army Institute of Surgical Research who require the use of enteral nutrition to meet all nutritional requirements are eligible for enrollment in this study.

Patient Inclusion. Ten patients meeting the following criteria are considered for entry into the study:

1. Male or female patients older than 18 years of age. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr of age and lack of menstrual periods > 1 yr), or have a negative pregnancy test.

2. Patients with burns > 30% of the total body surface area.

Patient Exclusion. Patients meeting the following criteria are excluded from the study:

1. Patients < 18 yr of age.

2. Patients with burns < 30% of the total body surface area.

3. Any pregnant patient.

4. Patients who have clinical and/or laboratory indications of sepsis. Any patient who develops sepsis during participation in the study are also excluded from the study at that time.

Procedures During the Study Period. On postburn day 10, each patient is transported to the Metabolic Room prior to the morning dressing changes. VO_2 and VCO_2 are measured utilizing the Horizon MMC Metabolic Cart™. The resting energy expenditure (REE) is calculated and baseline triglyceride, cholesterol, ketone, and insulin levels are obtained.

Each patient is started on enteral nutrition with energy requirements calculated as 1.2 x REE (13). Daily nitrogen requirements are calculated as 1 g/150 kcal. Carbohydrates are administered at a dose of 5 mg/kg/min. The remainder of kilocalorie requirement is administered as LCT. Electrolyte composition is tailored according to each individual patient's needs. Each patient receives standard vitamins and trace minerals. Once the patient's intake has reached his/her projected requirement for 3 continuous days, the VO_2, VCO_2, REE, nitrogen balance calculated from a 24-h UUN, and RQ are measured. Triglyceride, cholesterol, insulin, and ketone levels are obtained from a blood sample.

The following calculation is then made: ME (energy metabolism in kcal/min) = $(5.083 \times VO_2) + (0.138 \times VCO_2) - (0.125 \times NM)$. NM equals nitrogen metabolized in grams per minute calculated from the 24-h UUN.

Grams per minute of carbohydrate, fat, and protein metabolized are then calculated. The formulae used as previously described by Weir depends upon the RQ of the patient at each time.

The enteral formula is then changed. Protein and carbohydrate concentrations remain the same. Fat is administered as 90% MCT and 10% LCT. After a 3-day stabilization period, the same measurements are repeated.

The enteral formula is again altered. Protein remains the same, carbohydrates are reduced to 3.8 mg/kg/min, and fats consisting of 90% MCT and 10% LCT are then administered in sufficient quantity to meet the REE. The total amount of fat does not exceed 3 g/kg/day. After a 3-day stabilization period, all measurements are again repeated.

Upon completion of the study, the patient's enteral formula is changed back to a conventional formulation with 5 mg/kg/min carbohydrate and the residual caloric needs as 50% LCT and 50% MCT.

Statistical Analysis. The RQ, nitrogen balance, and percentage of calories from carbohydrate, fat, and protein will be compared by ANOVA for each patient.

RESULTS

Two patients have been enrolled in the study to date. These patients have both completed the study.

DISCUSSION

When the projected total of 10 patients have completed the study, the data will be analyzed as to the ability of

medium-chain triglycerides to serve as an effective energy source in thermally injured patients.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA313583	88 10 01	DD-DR&B(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3M161102BS14	IA	309
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code)
(U) Caloric Requirements of Thermally Injured Children

12. SUBJECT AREAS
06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 10	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
APPROVED BY

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	0.3	5
c. TYPE	d. AMOUNT	89	0.3	5
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BUESCHER, T M
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): CARLSON, D E
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available): MC MANUS, W F

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Thermal Injury; (U) Nutrition; (U) Metabolism; (U) Volunteers: (U) Children; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The caloric requirements of a burned child, imprecisely estimated by existing formulas, will be determined to optimize nutritional support. A literature search was performed and indicated no duplication of effort.

24. (U) On the fifth postburn day, the REE will be calculated as will the RQ. Baseline laboratory data will be collected and liver function and partial thromboplastin time tests will be performed. The patient will then be begun on alimentation that will be adjusted every 3 days until the caloric need is determined and met for two successive, 3-day cycle measurements as determined by a positive nitrogen balance, a RQ between 0.85 and 1.0, and a caloric intake equal to 1.25 times the REE.

25. (U) 8710 - 8809. This project was approved as minimal risk by the US Army Institute of Surgical Research Human Use Committee on 9 October 1987. No suitable pediatric patients have been admitted to the Institute during this reporting period. Patients will be asked to enroll in the study as they become available.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE. — U.S.G.P.O. 1986-491-003/30329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Caloric Requirements of Thermally Injured Children

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Teresa M. Buescher, MD, Captain, MC
William G. Cioffi, Jr., MD, Major, MC
Dawn E. Carlson, RD, Major, SP
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Caloric Requirements of Thermally Injured Children

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: Teresa M. Buescher, MD, Captain, MC
William G. Cioffi, Jr., MD, Major, MC
Dawn E. Carlson, RD, Major, MS
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

This project was approved by the US Army Institute of Surgical Research Human Use Committee on 9 October 1987. No suitable pediatric patients were admitted to the Institute during this reporting period. Patients will be asked to enroll in the study as they become available.

CALORIC REQUIREMENTS OF THERMALLY INJURED CHILDREN

The optimum nutritional support program for the thermally injured child has not been determined. The caloric requirements of a burned child are only marginally estimated by the existing formulas. The Curreri formulas, the Harris-Benedict Equations, and the Wilmore nomograms all differ in their estimation of the caloric requirements for children, e.g., a 2-yr-old girl weighing 12 kg (50th percentile) and measuring 86 cm in length (50th percentile) who has sustained a 40% total body surface area burn will have an estimated daily caloric requirement of 2,120 kcal by the original Curreri formula for children or 2,200 kcal by the Curreri "Junior" formula for 1- to 3-yr-olds, 1,839 kcal by the Harris-Benedict equation, and 1,600 kcal by the Wilmore nomograms. Determination of adequate nutritional support is important since inadequate caloric intake may result in protein wasting and malnutrition, whereas excess caloric intake can result in fatty infiltration of the liver, the fat intoxication syndrome, dehydration secondary to hyperglycemia and glucosuria, and excess carbon dioxide production with subsequent ventilator weaning failure. All of these potential problems could be avoided by the administration of the correct number of calories distributed between protein, fat, and carbohydrate.

Nitrogen requirements in thermally injured patients are increased over those in uninjured people. Numerous studies have demonstrated that injured, hypermetabolic patients demonstrate ineffective utilization of administered protein and have an optimum nitrogen to calorie ratio between 1:135 and 1:200 (grams nitrogen to nonprotein kilocalories). An optimum ratio of 1:150 has been recommended by Goodwin (3). Larger amounts of protein create a progressively more positive nitrogen balance but have not been shown to improve survival (7).

The role of fat as a source of nonprotein calories is dependent upon the extent of injury and the other nutrients administered. When diets lacking in protein are used, carbohydrate is more effective in sparing body protein than fat. However, when a "balanced" alimentation regimen containing protein, fat, and carbohydrate is devised, consideration is given to the administration of sufficient calories as fat not only to prevent essential fatty acid deficiency, but also to supply a large number of calories. Fat administration in excess of 3 g/kg/day in normal infants and 4 g/kg/day in normal adults can produce a fat overload syndrome (4). This has been described as consisting of hyperlipidemia, coagulopathy, fever, cholestatic jaundice, and gastrointestinal distress. This syndrome is believed to occur when the rate of infusion exceeds the maximum rate of peripheral clearance.

Studies comparing the utilization of fat and carbohydrate as energy sources have been undertaken in unburned surgical patients. A controlled study by MacFie et al (6) demonstrated that the administration of as little as 17% of calories as fat can reduce the loss of lean body tissue and the accumulation of body fat which is seen when glucose is used as the sole nonprotein energy source. A positive nitrogen balance has been achieved in postoperative patients with regimens supplying 33-38% of nonprotein calories from intravenous fat (9). The fat infusions depressed the respiratory quotient and insulin levels and they elevated the serum fatty acid and ketone levels, whereas the glucose infusions elevated the respiratory quotient and the pyruvate, lactate, alanine, and insulin levels. A RQ > 1.0 indicates that lipogenesis is occurring and that some of the administered calories are being utilized to synthesize fat (10).

The amount of glucose which can be effectively utilized by a stressed, injured patient is also unknown. Based on adult burn patients, Burke et al (1) have proposed that a value of 5 mg/kg/min is the maximum rate beyond which physiologically significant increase in protein synthesis and direct oxidation of glucose cannot be expected. At levels above this, there is increased carbon dioxide production and increased fatty infiltration of the liver. Looking at adult surgical patients, Hill and Church (5) have suggested a maximum rate of 7 mg/kg/min. However, neither of these studies addresses the situation of a burned child and the glucose administration ceiling remains unknown in this subpopulation of patients.

In a thermally injured child, these various formulas and recommendations create an impossible situation. Even when the lowest caloric estimate is used, the constraints of a 1:150 gram nitrogen to nonprotein kilocalories ratio, a maximum of 3 g/kg/day fat and a maximum of 5 mg/kg/min glucose are impossible to match. At least one of these recommendations must be ignored. The optimum nitrogen to kilocalorie ratio is well supported in the literature. The fat administration ceiling is well supported in unburned children but no data exist in burned children. The carbohydrate ceiling has also not been determined in burned children. For these reasons, the alimentation regimen which will be used as a starting point in this study will be based on the Wilmore nomograms for determination of the total caloric requirement. A 1:150 nitrogen to kilocalorie ratio will be maintained. The amount of fat will be initially limited to three g/kg/day and glucose will supply the remaining calories. It is expected that this glucose infusion rate may be > 5 mg/kg/min. If the patient is unable to tolerate the glucose infusion rate needed to deliver the calculated number of calories based on the initial estimate, the quantity of fat will be increased and the amount of carbohydrate decreased. This will continue until the total

number of calories delivered equals that suggested in the initial estimate. The quantity of lipid administered will be kept below that which causes a serum triglyceride level > 150 mg/dl. If it should prove to be impossible to reach the estimated caloric intake due to severe hyperglycemia and coexisting hyperlipidemia preventing further increase in both glucose and fat infusions, the oxygen consumption and carbon dioxide production will be determined at the maximum infusion rates which the patient will tolerate. These values shall be used as a starting point to calculate a more accurate measure of the caloric need. Further adjustments in the calories administered will follow these measurements and the respiratory quotient and resting energy expenditure determinations derived from these two values. The patient's caloric needs will be determined by measurements in the Metabolic Room using the Horizon™ metabolic cart and the nutritional support will be adjusted to administer kilocalories equal to 1.25 X REE (8), maintain the respiratory quotient between 0.85 and 1.00, and maintain a positive nitrogen balance. The amount of calories needed to comply with these restraints will be considered the patient's caloric requirement.

MATERIALS AND METHODS

Number of Patients. Twenty patients will be enrolled in the study. Properly signed signed and witnessed voluntary agreement affidavits will be obtained for each patient prior to enrollment in the study.

Inclusion Criteria. Patients meeting the following criteria will be eligible for enrollment in the study:

1. Patients admitted to the US Army Institute of Surgical Research with burn injury.

2. Male or female patients < 13 yr of age.

3. Patients with burn wounds > 30% of the total body surface area.

Exclusion Criteria. Patients meeting the following criteria will be excluded from enrollment in the study:

1. Patients 13 yr of age and older.

2. Patients with burn wounds < 30% of the total body surface area.

3. Patients with electrical injury.

4. Patients with fractures or major associated injuries.

5. Patients with inhalation injury.

6. Patients who are wards of the state or any other agency, institution, or entity.

Assent. For children from 6-12 yr of age, judgment by the primary investigator and the attending surgeon will be made as to whether the child is capable of assent. In determining whether the child is capable of assenting, the primary investigator and the attending surgeon will take into account the age, maturity, and psychological state of the child involved. This judgment will be made for each child. If it is deemed that the child is capable of assent, then the research protocol will be explained to that child in terms that he/she will understand. The child will then be enrolled in the study if his/her assent is given and permission is obtained from the child's parent or legal guardian. If it is deemed that the child is not capable of assent or if the child is 5 yr of age or younger, then permission will be obtained from the child's parent or legal guardian only.

Study Procedures. On the fifth postburn day, each patient will be transported to the Metabolic Room on Ward 14A prior to the morning dressing change. Oxygen consumption and carbon dioxide production will be measured using the Horizon™ metabolic cart. The environment temperature and humidity will be maintained constant throughout each patient's stay in the Metabolic Room. The REE will be calculated as will the RQ. Baseline laboratory data wil include serum electrolytes, creatinine, cholesterol, triglycerides, platelet count, prothrombin time, ketone, and insulin values. Liver function and partial thromboplastin time tests will also be performed. These serum laboratory values will be repeated at the time of each subsequent trip to the Metabolic Room for further measurements. All measurements in the Metabolic Room will take place prior to the morning dressing change. The patient's height and baseline weight will be determined upon admission. Weights will be obtained on a daily basis.

The patient will then be begun on alimentation using either parenteral hyperalimentation or enteral feeding. If possible, enteral feedings will be used to supply the patient's nutrition. If the patient's gastrointestinal tract is not capable of tolerating enteral feedings for any reason, intravenous hyperalimentation will be employed. The total calorie requirement will be based upon the lowest estimated caloric need as calculated from the Wilmore nomograms, the Curreri formulas, and the Harris-Benedict equations. Nitrogen administration will be calculated to produce a 1 g of nitrogen to 150 nonprotein kcal ratio. Lipids will be administered at a rate of 3 g/kg/day. Electrolyte composition of the fluids will be adjusted to the patient's needs. Each patient will receive standard vitamin and mineral supplements.

Once the patient's intake has reached the projected requirements and has remained stable for 3 days, the patient will be transported to the Metabolic Room where oxygen consumption and carbon dioxide production will again be measured. A 24-h urine collection will be obtained on that day as well. From this data, the RQ and REE will calculated. The grams of totally metabolized nitrogen, carbohydrate, and fat as well as the nitrogen balance will also be calculated.

Based on the new RQ, REE, and nitrogen balance measurements, the caloric requirements will be recalculated. If the RQ value is < 0.85, the total number of calories will be increased by 10%, maintaining the 1:150 gram of nitrogen to kilocalories ratio and the 3 g/kg/day lipid infusion rate. If the RQ is > 1.0, nitrogen, carbohydrate, and fat will be examined in an effort to determine which component or components (protein, carbohydrate, fat) should be reduced in order to decrease the total number of calories by 10% (2).

After a 3-day stabilization period, these metabolic measurements will be rechecked and again the caloric intake adjustd to bring the RQ to between 0.85 and 1.0 and to keep the nitrogen balance positive. This 3-day cycle will be repeated until the caloric need is determined and met for two successive, 3-day cycle measurements. This will be determined by a positive nitrogen balance, a RQ between 0.85 and 1.0, and a caloric intake equal to 1.25 X REE. Caloric needs shall be redetermined following any operative procedure after a 3-day stabilization period. During these days, alimentation will be maintained at the preoperative level.

RESULTS

This project was approved by the US Army Institute of Surgical Research Human Use Committee on 9 October 1987. No suitable pediatric patients were admitted to the Institute during this reporting period. Patients will be asked to enroll in the study as they become available.

DISCUSSION

When 20 patients have completed the study, the data will be analyzed to determine the optimum nutritional support program for the thermally injured child.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA314706	88 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
88 06 06	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	IA	310
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code)
(U) Salt and Water Balance in the Thermally Injured Patient

12. SUBJECT AREAS
06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
88 05	89 09	DA	C

MILITARY RELEVANCY CERTIFIED
APPROVED BY

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER				
c. TYPE	d. AMOUNT	88	0.1	5
e. KIND OF AWARD	f. CUM/TOTAL	89	0.1	7

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR CIOFFI, W G
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-4440
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (if available) BAUMAN, J M g. NAME OF ASSOCIATE INVESTIGATOR (if available) HARTSHONE, M R

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns; (U) Antidiuretic Hormone; (U) Atrial Natriuretic Peptide; (U) Renin-Angiotensin; Aldosterone Axis;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Resuscitation; (U) Blood Volume; (U) Renal Plasma Flow; (U) Volunteers: (U) Adults; (U) RAII

23. (U) The purpose of this study is to describe the alterations of plasma levels of antidiuretic hormone, atrial natriuretic peptide, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity. A literature search was performed and indicated no duplication of effort.

24. (U) Twenty consecutive patients will be entered into this study. On postburn days 2, 5, and 10, intravascular volume measurements will be made utilizing chromium-labeled RBCs to measure RBC volume. Also on postburn day 5, the glomerular filtration rate will be measured utilizing inulin and effective renal plasma flow will be measured using a colorimetric hippurate method.

25. (U) 8710 - 8809. This project was approved by the USAISR Research Council, the Brooke Army Medical Center Radiation Control Committee, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board and work is to be initiated shortly.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE * USGPO 1986-491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Salt and Water Balance in the Thermally Injured Patient

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Cheryl M. Crowley, MD, Captain, MC
Theresa A. Graves, MD, Captain, MC
Michael R. Hartshone, MD, Major, MC*
George M. Vaughan, MD, Lieutenant Colonel, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

*Nuclear Medicine Department
Brooke Army Medical Center
Fort Sam Houston
San Antonio, Texas 78234-6200

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Salt and Water Balance in the Thermally Injured Patient

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Cheryl M. Crowley, MD, Captain, MC
Theresa A. Graves, MD, Captain, MC
Michael R. Hartshone, MD, Major, MC*
George M. Vaughan, MD, Lieutenant Colonel, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Factors responsible for sodium and volume regulation following injury are not clearly understood. This study was designed to describe the alterations of plasma levels of antidiuretic hormone, atrial natriuretic peptide, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity. The information generated will permit refinement of fluid resuscitation regimens for severely burned and critically ill patients. To date, no patients have been enrolled in the study.

SALT AND WATER BALANCE IN THE THERMALLY INJURED PATIENT

Factors responsible for sodium and volume regulation following injury are not clearly understood. Several authors have interpreted their data to imply that resetting of hormonal control mechanisms occurs following thermal injury and that this is a stress response and not sodium and volume-dependent (1-2). Although various studies have examined one or two factors responsible for sodium and volume regulation following thermal injury, no one has studied the system as a whole. In normal man, antidiuretic hormone (ADH), atrial natriuretic peptide (ANP), and the renin-angiotensin-aldosterone loop are responsible for salt and water balance. How these systems interact following thermal injury is unknown.

The ADH response following thermal injury has been recently examined (2-4). Morgan et al have concluded that ADH levels are elevated postburn and remain so for 7-10 days. In addition, the increased ADH levels appear to have little relation to the serum osmolality and do not affect urine output. There is no satisfactory explanation for this at present. None of these studies have measured blood or plasma volume simultaneously with the measurements of ADH.

The renin-angiotensin-aldosterone axis has been examined in thermal injury (1). Shirani et al suggested that the elevated plasma levels of renin, angiotensin I, angiotensin II, and aldosterone following thermal injury reflect a resetting of hormonal control and are not dependent upon an effective plasma volume deficit. No volume measurements were made in this study. In this group of patients, combinations of these hormones did remain volume-responsive as verified by saline-loading tests.

ANP, a potent natriuretic and diuretic as well as a vasorelaxant agent, is present in mammalian cardiac atria (5). Central hypervolemia and increased blood pressure have been postulated as factors promoting ANP secretion (6). The vasorelaxant properties of ANP appear to be mediated by an increased level of intracellular cyclic guanosine monophosphate (GMP) which antagonizes the vasopressor effects of angiotensin II and norepinephrine (6). Infusion of ANP in humans effects a profound natriuresis with an accompanying diuresis (7). The mechanism by which ANP causes these changes in unclear. In animal models, infusion of ANP causes an increase in the glomerular filtration rate (GFR) as well as sodium excretion (8). It appears, at least in part, that the mechanism responsible for natriuresis is the increase in GFR. No proximal tubular effect of ANP has been documented. The renal effects of ANP can be blocked by calcium channel blockers, suggesting that its effects are calcium-dependent (8).

ANP also has an effect on ADH release and the renin-angiotensin-aldosterone axis. In isolated rat posterior pituitary lobes, ANP causes a massive release of ADH (9). Other investigators have indicated that in isolated hypothalamic hypophyseal preparations, ANP induces a decrease in ADH. ANP induction of ADH release may serve as a negative feedback loop regulating the actions of ANP. The effects of ANP on the renin-angiotensin-aldosterone axis appear to be more constant. Infusion of ANP causes a decrease of plasma renin activity and renin excretion (10,12) and at the same time blunts aldosterone release stimulated by angiotensin II (11-12).

A syndrome of inappropriately low plasma aldosterone levels in the presence of elevated plasma renin activity has been identified in a subset of critically ill patients and was associated with a higher mortality during critical illness (13). The nature of this abnormality has not been elucidated. Elevated ANP could explain this dissociation during critical illness, with its ability to decrease aldosterone levels in the face of an activated renin system (14).

The effects of thermal injury on plasma ANP level and how it in turn affects salt and water balance have not been described. The purpose of this study will be to describe the alterations of plasma levels of ADH, ANP, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity. The information generated will permit refinement of fluid resuscitation regimens for severely burned and critically ill patients.

MATERIALS AND METHODS

Number of Patients. Twenty consecutive patients admitted to the US Army Institute of Surgical Research will be eligible for enrollment in this study.

Inclusion Criteria. Patients meeting the following criteria will be eligible for enrollment in the study:

1. Male or female patients > 18 yr of age. Female patients be previously surgically sterilized or postmenopausal (> 45 yr of age and lack of menstrual periods for > 1 yr) or have a negative pregnancy test.

2. Patients with burns between 30% and 80% of the total body surface area.

3. Patients admitted to the US Army Institute of Surgical Research within 24 h of the time of injury.

Exclusion Criteria. Patients meeting the following criteria will be excluded from the study:

1. Patients < 18 yr of age.

2. Any pregnant patient.

3. Patients with burns < 30% or > 80% of the total body surface area.

4. Patients admitted to the US Army Institute of Surgical Research > 24 h postburn.

5. Patients with a history of diabetes mellitus or congestive heart failure.

6. Patients with a history of treatment for hypertension within the previous month.

7. Patients with concomitant CNS injury.

8. Patients with sepsis or who develop sepsis during the study period.

9. Patients with acute renal failure or who develop acute renal failure during the study period (defined as an acute rise in serum creatinine to a level > 1.5).

Patient Procedures During the Study Period.

Part I. Upon enrollment into the study, the following data will be collected each day for each patient on postburn days 2-10:

1. Percentage of the total body surface area burned.

2. Medications administered.

3. Body weight.

4. Total intake of water and salt.

5. Urine and nasogastric output, to include volume as well as sodium and potassium content.

6. Serum concentrations of sodium, potassium, chloride, glucose, phosphate, uric acid, urea nitrogen, creatinine, and beta$_2$-microglobulin.

7. Serum and urine osmolality.

8. Urine concentrations of creatinine, urea nitrogen, phosphate, total protein, beta$_2$-microglobulin, and aldosterone from a 24-h urine sample.

From this data, the endogenous GFR, osmolality clearance, $H_2O(CH_2O)$ clearance, and fractional excretion of sodium will be calculated.

At 0700 h each morning at the time of the routine blood drawings, blood will be obtained for ADH, ANP, plasma renin activity, and aldosterone assays.

Part II. On postburn days 2, 5, and 10, intravascular volume measurements will be made utilizing chromium-labeled RBCs to measure RBC volume. Total blood volume will then be calculated after measuring a central hematocrit.

On postburn day 5, a Swan-Ganz catheter, if not already in place, will be inserted through the central line which the patient will already have for clinical care and readings of cardiac output and pulmonary artery occlusion pressures will be recorded. Systemic vascular resistance will be calculated from the appropriate variables.

Also on postburn day 5, GFR will be measured utilizing the inulin technique. Effective renal plasma flow will be measured using a colorimetric hippurate study. Inulin clearance will be repeated for any patient who demonstrates a subsequent decrease in renal function during the hospital course.

RESULTS

No patients have been enrolled in the study to date.

DISCUSSION

When 20 patients have completed the study, the data will be analyzed as to the alterations of plasma levels of ADH, ANP, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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11. Anderson JV, Struthers AD, Payne NN, et al: Atrial natriuretic peptide inhibits the aldosterone response to angiotensin II in man. **Clin Sci** 70:507-12, 1986.

12. Maack T, Marion DN, Camargo MJ, et al: Effects of auriculin (atrial natriuretic factor) on blood pressure, renal function, and the renin-aldosterone system in dogs. **Am J Med** 77:1069-75, 1984.

13. Findling JW, Waters VO, and Hershel R: The dissociation of renin and aldosterone during critical illness. **J Clin Endocrinol Metab** 64:592-5, 1987.

14. Weidmann P, Hellmueller B, Uehlinger DE, et al: Plasma levels and cardiovascular, endocrine, and excretory effects of atrial natriuretic peptide during different

sodium intakes in man. J Clin Endocrinol Metab 62:1027-36, 1986.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA315349	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	DA	311
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

12. SUBJECT AREAS
06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 01	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

a. DATE EFFECTIVE APPROVED BY [signature]

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.0	0
89	0.2	8

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Epidermal Growth Factor; (U) Fibroblast Growth Factor; (U) Platelet-Derived Growth Factor; (U) Epithelization;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Thermal Injury; (U) Lab Animals: (U) Guinea Pigs; (U) RAII

23. (U) The purpose of this study is to determine whether epidermal growth factor, fibroblast growth factor, or platelet-derived growth factor can enhance burn epithelization in a partial-thickness burn wound in guinea pigs. A literature search was performed and indicated no duplication of effort.

24. (U) After receiving deep partial-thickness burns, male guinea pigs will be divided into four treatment groups. Group I will receive 0.5 cc lanolin cream applied to the burn wound twice daily. Groups II, III, and IV will receive, in similar fashion, 0.5 cc lanolin cream to which epidermal growth factor, fibroblast growth factor, or platelet-derived growth factor has been added. At the time of sacrifice, the extent of healing by contraction will be assessed utilizing a planimeter and the extent of epithelization will be assessed histologically.

25. (U) 8710 - 8809. This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 14 January 1987. When a suitable source for the growth factors has been identified, the project will begin. This project was transferred from DA312335.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE. — * USGPO 1986-491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA315350	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	DA	312
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Cellular Host Defense Function After Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells

12. SUBJECT AREAS
06 01 Biochemistry 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 05	90 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Basil A. Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.0	0
89	2.0	60

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BURLESON, D G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-7138
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): MASON, A D
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Flow Cytometry; (U) Lymphocyte Subpopulations; (U) Burn Injury; (U) Infection; (U) Immunocompetence;

23 TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Volunteers; (U) Adults; (U) ILIR; (U) RAII

23. (U) To analyze the complex leukocyte mixtures seen in the blood of burned patients, quantitate the changes that occur, and correlate those changes with changes in cell function as well as clinical outcome. A literature search was performed and indicated no duplication of effort.

24. (U) The immune status of burn patients will be assessed in terms of lymphocyte subpopulation composition and function using flow cytometry to differentiate the subpopulations. The data will be correlated with patient mortality and morbidity and compared to data from unburned controls.

25. (U) 8710 - 8809. Data was collected and analyzed for 69 patients. Data indicate the proportion of T lymphocytes, including helper and suppressor subsets, decreased in burn patients as compared to control subjects. The proportion of B lymphocytes increased, but there was no change in NK cells. The ratio of helper to suppressor lymphocytes was also unchanged in burn patients. Nonsurviving patients had decreased proportions of helper and suppressor lymphocytes, yet the proportion of T lymphocytes was unchanged as compared to survivors. Nonsurviving patients also had decresad T lymphocyte function as compared to survivors. This decrease in function was specific for T lymphocytes as B lymphocyte function remained unchanged or increased. This project was transferred from DA 311488.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA315351	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&B(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	DA	313
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) A Study of Biochemical Changes in the Cellular Environment of Tissue of the in vivo Partial-Thickness Rat Burn Wound

12. SUBJECT AREAS
06 01 Biochemistry 06 04 Anatomy and Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 09	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Basil A. Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.0	0
89	1.1	55

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR BROWN, W L
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-4652
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) MASON, A D
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn Wound Metabolism; (U) Edema; (U) Burn Injury; (U) Lab Animals; (U) Rats; (U) RAII

23 TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

23. (U) Determination of the biochemical and metabolic changes that occur in the in vivo partial-thickness rat burn wound during the early postburn period and identification of criteria of reversibility. The data generated may identify means to block or reverse the metabolic changes and limit the progression in the extent and severity of injury in wounds of burned soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) Microelectrodes will be used to measure changes in extracellular potassium ion content and in pH and/or carbon dioxide partial pressure at various sites in the in vivo burn wound. Samples from sites adjacent to the microelectrodes will be taken to measure selected metabolites using enzymatic methods. Cells and subcellular organelles will be isolated for measurement of changes in function with time postburn.

25. (U) 8710 - 8809. We have completed the series of measurements of water content, dry weight, and pH of 20% sham and partial-thickness scald burn wounds from 1-72 h postburn. We are currently testing methods for separating epidermal and dermal cells to be used in studies of the effect of tissue pH and time postinjury on cell function. This project was transferred from DA311489.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * U.S.G.P.O. 1988 -491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA315353	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	F	314
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) The Effect of Interleukin-2 Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

12. SUBJECT AREAS
06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 10	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Basil A. Pruitt Jr.

a. DATE EFFECTIVE

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.0	0
89	0.4	27

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR CIOFFI, W G
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-4440
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Interleukin-2; (U) Pseudomonas; (U) Sepsis; (U) Burns; (U) Lab Animals: (U) Rats; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The effects of exogenous interleukin-2 administration following burn wound infection will be studied in a rodent model. A literature search was performed and indicated no duplication of effort.

24. (U) Two iterations of the study were performed to determine the optimal dose of interleukin 2 and the optimal postburn day for administration. Upon completion of these studies, two addenda were submitted and approved. The project will be continued with the addition of indomethacin in high and low doses administered concomitant with interleukin 2 in an attempt to increase interleukin 2 receptor expression.

25. (U) 8710 - 8809. The optimal dose which could be tolerated by the septic animal was identified. In LD100 and LD50 models, interleukin 2 administration concomitant with burning and seeding of the burn wound with Pseudomonas failed to result in improved survival. To increase interleukin 2 receptor expression, both high doses (5 mg/kg) and low doses (0.5 mg/kg) of indomethacin were administered with the interleukin 2. This failed to improved survival in the LD100 model. This project was transferred from DA313322.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * USGPO 1986-491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA315354	88 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	IA	315
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Development of Thermal Ionization Mass Spectrometry Methodology for the Study of Calcium Metabolism

12. SUBJECT AREAS
06 01 Biochemistry 06 04 Anatomy and Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
88 05	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

a. DATE EFFECTIVE APPROVED BY

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.0	0
89	0.3	21

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIPPEE, R L
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-7138
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): VAUGHAN, G M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available): OKERBERG, C V

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns; (U) Mass Spectrometry; (U) Metabolism; (U) Homeostasis; (U) Lab Animals; (U) Rats; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The purpose of this study is to develop thermal ionization mass spectrometry techniques to investigate the homeostatic mechanisms of calcium regulation after thermal injury. Methodology developed from this study will be applied to clinical studies of calcium metabolism in thermally injured soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) Male Sprague-Dawley rats will be used as a burn model to study the effect of burn injury on calcium homeostasis. Stable isotopes of calcium will be used to perform calcium kinetic analyses after burn injury. Total fecal and urine excretion will be collected to perform calcium balance calculations. The combined data from the kinetic analyses and the balance calculations will be analyzed using a mathematical modeling computer program to determine the effect of burn injury on the various aspects of calcium metabolism.

25. (U) 8805 - 8809. This project was approved by the USAISR Research Council and the US Army Institute of Surgical Research Animal Care and Use Committee during April 1988. Equipment and supplies have been ordered and work will be initiated upon their arrival. This project was transferred from DA314599.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * USGPO 1986-491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA315355	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CA	316
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) The Effect of High Frequency Ventilation on VA/Q in Sheep with Inhalation Injury

12. SUBJECT AREAS
06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 01	89 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.0	0
89	0.2	7

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Inhalation Injury; (U) High Frequency Ventilation; (U) Ventilation-Perfusion Ratio; (U) Cardiac Output; (U) Lab

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Animals: (U) Sheep; (U) RAII

23. (U) To compare the effects of volumetric diffusive ventilation and conventional ventilation on pulmonary and hemodynamic indices which are altered in an ovine inhalation injury model. A literature search was performed and indicated no duplication of effort.

24. (U) Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this Institute. Animals will be randomized to treatment with either conventional or high frequency ventilation. Changes in VA/Q as well as other pulmonary and physiologic measurements will be compared between groups.

25. 8710 - 8809. (U) This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 14 January 1987. Due to technical problems with the newly acquired mass spectrophotometer, the multiple inert gas elimination technique has not been operational. When the technique has been revalidated, the study will continue. This study was transferred from DA312336.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE. — USGPO 1986-491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA315356	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	AZ	317
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP.	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

12. SUBJECT AREAS
06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 01	90 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
APPROVED BY [signature]

17. CONTRACT/GRANT	
a. DATE EFFECTIVE	
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.0	0
89	0.6	40

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): MASON, A D
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available): OKERBERG, C V

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Shock; (U) Resuscitation; (U) Hemodynamics; (U) Plasmaphoresis; (U) Fluid Replacement; (U) Albumin;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Crystalloids; (U) Lab Animals: (U) Sheep; (U) RAII

23. (U) To determine the hemodynamic consequences of controlled pure plasma loss in sheep using a method to simulate the acute burn. Subsequently, response to therapy will be assessed in burned soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) A plasmaphoresis filter will be used to produce intravascular plasma loss similar to that caused by burn injury. This device selectively removes plasma while leaving the formed elements of blood in the vascular system.

25. (U) 8710 - 8809. Eight animals were studied during this reporting period and the model has been established in a reliable fashion. Animals subjected to pure plasma loss have been resuscitated with several resuscitation schema, including crystalloid and colloid fluids. Data from these 8 animals are currently being analyzed. An addendum is being written which will validate the model by comparing the hemodynamic changes seen in a 50% burn model with those seen in the pure plasma volume loss model. This project was transferred from DA312334.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE. * U.S.G.P.O. 1986-491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA315357	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	F	318
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Antibacterial and Wound Healing Effects of Silver-Nylon Electrodes with Weak Direct Current

12. SUBJECT AREAS
06 01 Biochemistry 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
88 08	90 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
APPROVED BY [signature]

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE		FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	0.0	0
c. TYPE	d. AMOUNT	89	1.0	50
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CHU, C S
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-3411
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): MC MANUS, A T
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available): OKERBERG, C V

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Silver; (U) Silver-Nylon Cloth; (U) Skin Healing; (U) Skin Grafts; (U) Direct Current; (U) Burns; (U) Lab Animals:

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (U) Guinea Pigs; (U) Rats; (U) RAII

23. (U) To determine the antimicrobial and wound healing effects of weak direct current and to measure silver concentrations of burn eschars and underlying tissue as a function of time and direct current. If accelerated healing of burn wound and donor sites is achieved, the treatment time for burned soldiers could be reduced. A literature search was performed and indicated no duplication of effort.

24. (U) The guinea pig will be used to study healing in deep second degree burns, third degree burns, donor site wounds, and skin grafts with and without stimulation with weak direct current. The extent of fibrosis, collagen accumulation, and depth of neovasculature will be measured by examination of special stain preparations. Wound contracture will be measured planimetrically. Silver concentrations of burn eschars and important internal organs will be determined by atomic absorption technology in the rat model. Data will be analyzed as multiway frequency tables with Chi square, Yates corrected Chi square, or Fisher's exact test comparisons.

25. (U) 8808 - 8809. This project was approved by the USAISR Research Council and the US Army Institute of Surgical Research Animal Care and Use Committee during August 1988 and work will be initiated within the next few weeks.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * USGPO 1986 -491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA312335	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	K	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	075
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

12. SUBJECT AREAS
06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 01	88 09	DA	C

MILITARY RELEVANCY CERTIFIED
APPROVED BY

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	0.2	5
c. TYPE	d. AMOUNT	89	0.0	0
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR CIOFFI, W G
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-4440
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (if available) g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Epidermal Growth Factor; (U) Fibroblast Growth Factor; (U) Platelet-Derived Growth Factor; (U) Epithelization;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Thermal Injury; (U) Lab Animals: (U) Guinea Pigs; (U) RAII

23. (U) The purpose of this study is to determine whether epidermal growth factor, fibroblast growth factor, or platelet-derived growth factor can enhance burn epithelization in a partial-thickness burn wound in guinea pigs. A literature search was performed and indicated no duplication of effort.

24. (U) After receiving deep partial-thickness burns, male guinea pigs will be divided into four treatment groups. Group I will receive 0.5 cc lanolin cream applied to the burn wound twice daily. Groups II, III, and IV will receive, in similar fashion, 0.5 cc lanolin cream to which epidermal growth factor, fibroblast growth factor, or platelet-derived growth factor has been added. At the time of sacrifice, the extent of healing by contraction will be assessed utilizing a planimeter and the extent of epithelization will be assessed histologically.

25. (U) 8710 - 8809. This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 14 January 1987. When a suitable source for the growth factors has been identified, the project will begin. This project was transferred to DA315349.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE. — * U.S.G.P.O. 1988 -491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Chi-Sing Chu, MD
Carlin V. Okerberg, DVM, PhD, Major, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Chi-Sing Chu, MD
Carlin V. Okerberg, DVM, PhD, Major, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

The ability of various growth factors to enhance wound healing has received recent interest with the advent of recombinant DNA techniques. Utilizing this technology, increased quantities of various factors previously available only in extremely small amounts are now being used for study. The purpose of this study is to determine whether these three growth factors can enhance burn epithelization in a partial-thickness burn wound in guinea pigs. However, a suitable source for the procurement of growth factors has not been found.

EFFECT OF GROWTH FACTORS ON THE HEALING OF PARTIAL-THICKNESS SCALD WOUNDS IN THE GUINEA PIG

The ability of various growth factors to enhance wound healing has received recent interest with the advent of recombinant DNA techniques. Utilizing this technology, increased quantities of various factors previously available only in extremely small amounts are now being used for study. Epidermal growth factor (EGF), initially isolated from the submaxillary gland of mice (1) and subsequently identified in human urine (2), has been shown to increase the rate of endothelial and epithelial proliferation (3). The mitogenic effects of EGF have been documented in several models (4-5), although its effectiveness in stimulating epithelization in burn wounds has not been documented (6-7). Fibroblast growth factor (FGF), originally noted for its mitogenic effect on fibroblast, has recently been found to have potent angiogenic properties. Platelet-derived growth factor (PDGF) appears to have a variety of properties, one of which is stimulation of epithelization.

The purpose of this study is to determine whether these three growth factors can enhance burn epithelization in a partial-thickness burn wound in guinea pigs. If growth factors can favorably alter the course of burn wound healing in this model, it will form the scientific basis for further investigations of tissue growth factors.

MATERIALS AND METHODS

Study Design. Male guinea pigs weighing 400-500 g will be anesthetized with sodium pentobarbital (35 mg/kg IP). The dorsal surface will be shaved and a 20% partial-thickness scald injury produced. Animals will be secured to specially constructed template devices and the exposed dorsal surfaces exposed to 90°F water for 5 sec to acuate a deep partial-thickness burn (8). Upon completion of burning, the burn wound edges will be tattooed and the animals will be allowed to recover from anesthesia. They will then be housed in individual cages and fed food and water *ad libitum* throughout the study period. Four groups of 40 animals each will be studied. Group I will serve as the control group, Group II will receive EGF, Group III will receive FGF, and Group IV will receive PDGF. Group I animals will receive 0.5 cc lanolin cream (Squibb-Novo, Inc., Princeton, NJ) applied to the burn wound twice daily. Group II will receive 0.5 cc EGF in a lanolin base (10 µg/ml) twice daily. Groups III and IV will receive FGF and PDGF, respectively, prepared in a similar manner. Wounds will be measured daily for assessment of contraction. This will be accomplished by measuring the burn wound area utilizing the tattoo mark placed at the time of burning. On postburn days 5 and 10, 5 animals in each group

will be sacrificed and 15 animals in each group will be sacrificed on postburn days 20 and 30.

Histological Evaluation. At the time of sacrifice, the extent of healing by contraction will be assessed utilizing a planimeter and the extent of reepithelization will be assessed histologically. Tissues will be taken for evaluation of the general health of the animal and evaluation of concurrent disease. Full-thickness skin sections will be taken at the burn margin (to include burned and nonburned skin) to evaluate the healing process. Electron microscopy will be peformed as indicated. All tissues will be preserved, processed, and cut using standard methods.

Statistical Analysis. Data will be analyzed by ANOVA.

RESULTS

This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 14 January 1987. However, a suitable source for the procurement of growth factors has not been found.

DISCUSSION

When a suitable source for the procurement of growth factors has been identified, this study will continue.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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2. Starkey RH, Cohen S, and Orth DN: Epidermal growth factor: Identification of a new hormone in human urine. **Science** 189:800-2, 1975.

3. Gospodarowicz D, Mescher AL, and Birdwell CR: Stimulation of corneal endothelial cell proliferations in vitro by fibroblast and epidermal growth factors. **Exp Eye Res** 25:75-89, 1977.

4. Cohen S and Carpenter G: Human epidermal growth factor isolation and chemical and biological properties. **Proc Natl Acad Sci USA** 72:1317-21, 1975.

5. Cohen S and Elliott GA: The stimulation of epidermal keratinization by a protein isolated from the submaxillary gland of the mouse. **J Invest Derm** 40:1-5, 1963.

6. Thornton JW, Hess CA, Cassingham V, and Bartlett RH: Epidermal growth factor in the healing of second degree burns: A controlled animal study. **Burns** 8:156-60, 1981-1982.

7. Arturson G: Epidermal growth factor in the healing of corneal wounds, epidermal wounds, and partial-thickness scalds: A controlled animal study. **Scand J Plast Reconstr Surg** 18:33-7, 1984.

8. Walker HL and Mason AD Jr: A standard animal burn. **J Trauma** 8:1049-51, 1968.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA311488	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	K	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	076
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Cellular Host Defense Function After Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells

12. SUBJECT AREAS

06 01 Biochemistry 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 05	88 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY [signature]
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.5	50
89	0.0	0

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BURLESON, D G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-7138
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Flow Cytometry; (U) Lymphocyte Subpopulations; (U) Burn Injury; (U) Infection; (U) Immunocompetence;

23 TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Volunteers: (U) Adults; (U) ILIR; (U) RAII

23. (U) To analyze the complex leukocyte mixtures seen in the blood of burned patients, quantitate the changes that occur, and correlate those changes with changes in cell function as well as clinical outcome. A literature search was performed and indicated no duplication of effort.

24. (U) The immune status of burn patients will be assessed in terms of lymphocyte subpopulation composition and function using flow cytometry to differentiate the subpopulations. The data will be correlated with patient mortality and morbidity and compared to data from unburned controls.

25. (U) 8710 - 8809. Data was collected and analyzed for 69 patients. Data indicate the proportion of T lymphocytes, including helper and suppressor subsets, decreased in burn patients as compared to control subjects. The proportion of B lymphocytes increased, but there was no change in NK cells. The ratio of helper to suppressor lymphocytes was also unchanged in burn patients. Nonsurviving patients had decreased proportions of helper and suppressor lymphocytes, yet the proportion of T lymphocytes was unchanged as compared to survivors. Nonsurviving patients also had decresad T lymphocyte function as compared to survivors. This decrease in function was specific for T lymphocytes as B lymphocyte function remained unchanged or increased. This project was transferred to DA315351.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE • USGPO

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Cellular Host Defense Function after Thermal Injury: Assessment by Flow Cytomery of Peripheral Blood Cells

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

David G. Burleson, PhD, Lieutenant Colonel, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Cellular Host Defense Function after Thermal Injury: Assessment by Flow Cytomery of Peripheral Blood Cells

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: David G. Burleson, PhD, Lieutenant Colonel, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Analysis of circulating lymphocyte subpopulations from burn patients revealed major changes occurred after injury. Burn patients were leukocytotic but lymphopenic, which made isolation and measurement of lymphocyte subpopulations difficult. Ficoll-Hypaque gradients, flow cytometer light scatter gating, and a monoclonal antibody that marked contaminating cells were used to increase accuracy. Both helper (CD4 positive) and suppressor/cytotoxic (CD8 positive) lymphocytes were decreased after injury in comparison to unburned control subjects. Burn patients who died had greater decreases in their T lymphocyte subpopulations than survivor burn patients. The proportions of B, HLADR positive, and null cells were increased in burn patients compared to unburned control subjects while there were no differences in the proportion of Leu-8 positive, transferrin receptor positive, and large granular lymphocytes compared to control subjects. The ratio of CD4 positive to CD8 positive lymphocytes was the same for burn patients and control subjects, but the ratio was higher in burn patients who died compared to those who survived.

CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMERY OF PERIPHERAL BLOOD CELLS

Increased susceptibility to opportunistic infection continues to be a major problem in the treatment of burned patients. The basis for the increased susceptibility remains an enigma. Many cellular aspects of host defense have been measured in burned patients and found to be abnormal, yet none of the abnormalities have been clearly associated with the increased susceptibility to infection. A detailed analysis of cellular components of host defense may be necessary before the nature of the underlying defect can be determined and an effective treatment devised.

Several reports on changes in lymphocyte phenotype after thermal injury have established that subpopulation changes occur in the peripheral blood of human patients (1-4). However, the exact nature of these changes still remains controversial since considerable differences exist between the data presented in each of the reports. In addition, several new monoclonal antibodies have become available that are able to more clearly define lymphocyte subpopulations than those used in the previous studies. We here present a new report on the changes that occur in lymphocyte subpopulations in burned patients. The data was obtained from a relatively large patient population using an extensive panel of monoclonal antibodies to various subpopulation surface markers.

MATERIALS AND METHODS

Study Population Data. Data from 33 burn patients were compared with that from 33 control subjects. All patients (3 females and 30 males) were entered into the study within 5 days postburn. Admission to the study required that the patient's expected mortality (determined from burn size and age), based on previous experience at this Institute, be between 20% and 90% (average 55.4%). The average burn size was 46.5% and the average age was 43.4 yr. The actual mortality experienced by this group of patients was 32.5%. Control subjects were healthy individuals comprised of laboratory and hospital staff. The average age for this group was 40 and there were 10 females and 23 males.

Cell Preparations. Blood samples with EDTA as anticoagulant were obtained from burn patients 2X weekly for up to 8 wk postburn. Blood samples were taken at random from the 33 control subjects. Lymphocytes were separated from RBCs and nonlymphoid cells by Ficoll-Hypaque gradients. The cells isolated from the gradients were washed 3X in HBSS and resuspended for counting with a Coulter counter (Model ZM, Coulter Electronics, Inc., Hialeah, FL). Cell concentrations were adjusted to 1×10^7 for staining with monoclonal

antibodies. Since lymphocyte preparations from burn patients can be significantly contaminated with nonlymphoid cells, a slide was prepared on a cytocentrifuge using a portion of each gradient purified sample to aid in determining the extent of contamination.

Cell Staining. Cells were stained with the appropriate monoclonal antibodies, either chemically bound to phycoerythrin or fluorescein isothiocyanate or unconjugated in combination with goat-anti-mouse IgG (Fab2') conjugated to Texas Red (TAGO). The monoclonal antibodies were purchased from Becton Dickinson (Mountain View, CA). The antibodies used were anti-Leu-2 (CD8 T suppressor/cytotoxic subpopulation), anti-Leu-3 (CD4 T helper/inducer subpopulation), anti-Leu-4 (CD3 T lymphocyte receptor), anti-Leu-12 (CD19 B lymphocyte), anti-HLADR, anti-Leu-7 (large granular lymphocyte), anti-Leu-8 (a monoclonal binding to the surface of a subset of helper and suppressor lymphocytes as well as some B cells, monocytes, and neutrophils), anti-Leu-11 (CD16 NK cell or IgG Fc receptor), anti-Leu-15 (CD11b complement receptor III) anti-transferrin receptor, and anti-Leu-M3 (CD14 monocyte). IgG_1 or IgG_2 conjugated with the appropriate dye marker was employed as an isotypic control. The staining procedure followed that specified by the manufacturer of the monoclonal antibody. Cells were fixed immediately after staining in 1% paraformaldehyde.

Flow Cytometry Analysis. Subpopulations were analyzed by flow cytometry using either a FACS (Model 435, Becton Dickinson) or an EPICS (Model 753, Coulter Electronics, Inc.) flow cytometer. Electronic gates were set on forward angle and side scatter intensity using normal human peripheral blood lymphocytes. This gate was used to to exclude as many nonlymphoid cells as practical. Nonlymphoid cell contamination was monitored by observing the level of anti-Leu-M3 positives (anti-Leu-M3 binds monocytes and weakly binds granulocytes). The positive cutoff was set at a point that defined 1% or less of the electronically gated isotypic control sample as positive.

Data Analysis. Group means for data that were normally distributed were compared by t test (Program 7D, BMDP Statistical Software, San Francisco, CA). Data that were not normally distributed were compared by nonparametric analysis (Program 3S, BMDP Statistical Software).

RESULTS

Leukocyte Counts. A WBC count and a differential were performed on each specimen. Burn patient average WBC counts increased above that of control subjects soon after injury and remained elevated during the postburn course (Fig 1). Though WBC counts were increased, the mean lymphocyte counts

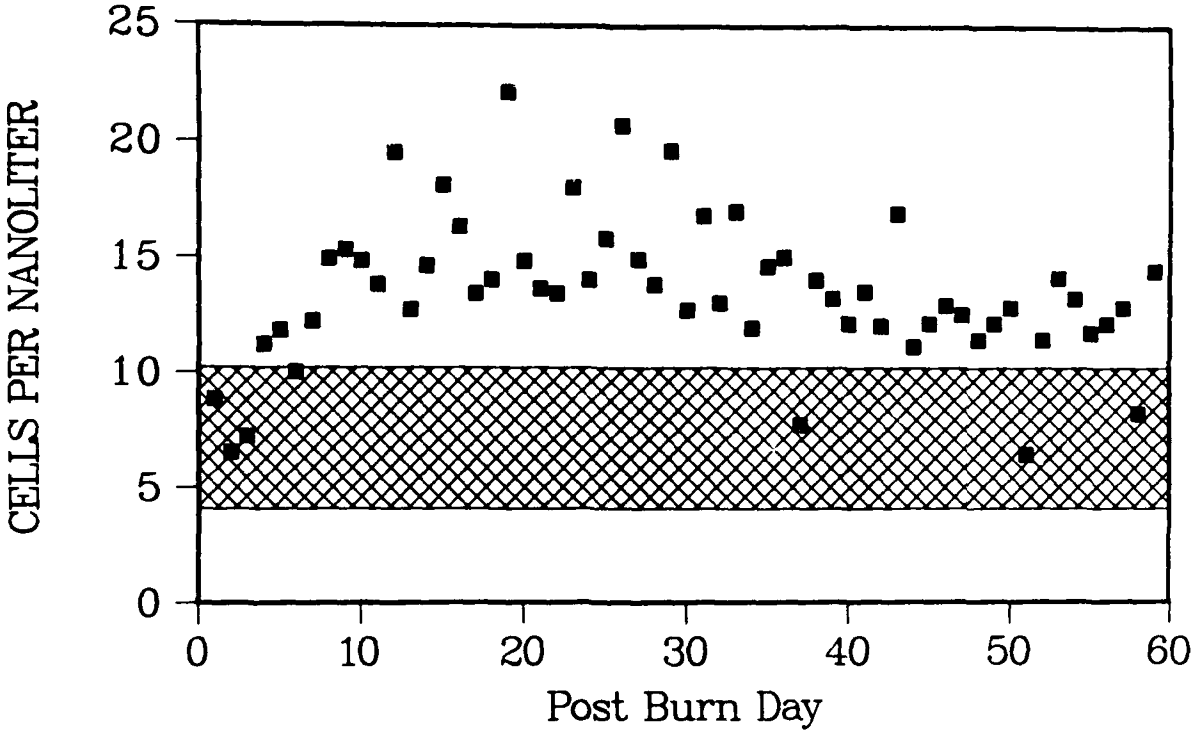

FIGURE 1. WBC counts in burn patients. The average WBC counts for all patients is displayed vs. postburn day. Control subject values ± 1SD are represented by the shaded horizontal bar.

determined by the Wright's stain differential were reduced in burned patients compared to control subjects (Fig 2). This lymphopenia, in combination with leukocytosis, makes isolation of lymphocytes for analysis more difficult. Ficoll-Hypaque gradient purification results in an average four-fold increase in the proportion of lymphocytes for analysis, but significant contamination remains (Fig 3). Selective electronic gating on the flow cytometer was required to insure that a high percentage of lymphocytes were analyzed. Electronic gating alone on lysed whole blood does not adequately eliminate contaminating nonlymphoid cells (5).

Lymphocyte Subpopulations. Ficoll-Hypaque purified samples obtained from all participants were analyzed for lymphocyte subpopulations. Burn patients had decreased proportions of T lymphocytes, including both helper and suppressor subsets (Fig 4) compared to control subjects. Since the proportion of T lymphocyte subsets was decreased in burn patients, the percentage of non-T subpopulations (B lymphocytes and NK cells) should have increased if their absolute number remained constant. In fact, B lymphocyte proportions increased while

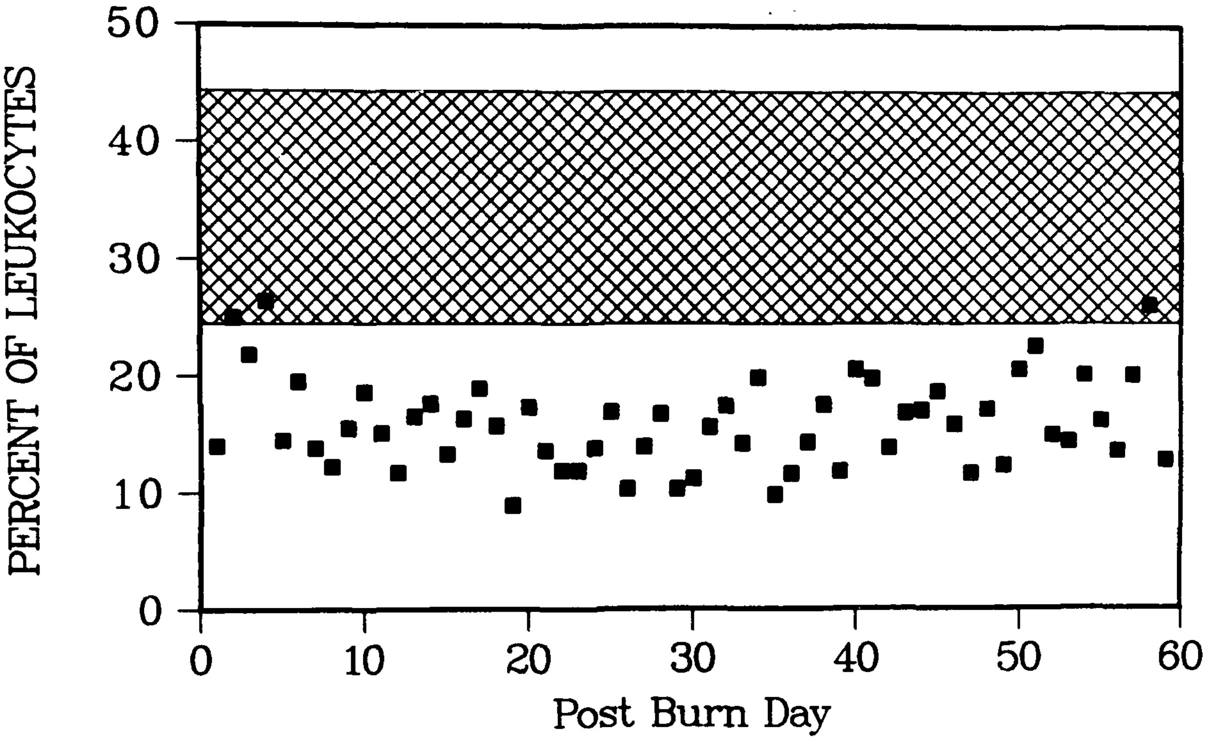

FIGURE 2. Whole blood lymphocyte counts in burn patients. The average whole blood lymphocyte count determined from the Wright's stain differential for all patients is displayed vs. postburn day. Control subject values ± 1SD are represented by the horizontal bar.

the proportion of NK cells was unchanged compared to control subjects.

The increase in the proportion of B lymphocytes did not make up the difference caused by the decrease in T lymphocytes. The combined percentages of pan T positive cells, B cells, NK cells, and monocytes for each sample should approach 100% as these monoclonal antibodies are directed at different subpopulations. Any cells not included in these subpopulations would be "null" cells or cells of unknown phenotype. The percentages determined for each of the populations were combined to reconstruct the analysis sample. This sum was 85 ± 23.6% for control subjects but only 71.6 ± 26.9% for burn patients ($P < 0.001$). Thus, there was an increase in "null" cells that were not bound by the monoclonal antibodies used in this panel. This increase could be the result of an increase in cells that have a antigen surface density so low that they could not be detected by the techniques used or it could represent an influx of subpopulations of unknown phenotype not bound by these antibodies.

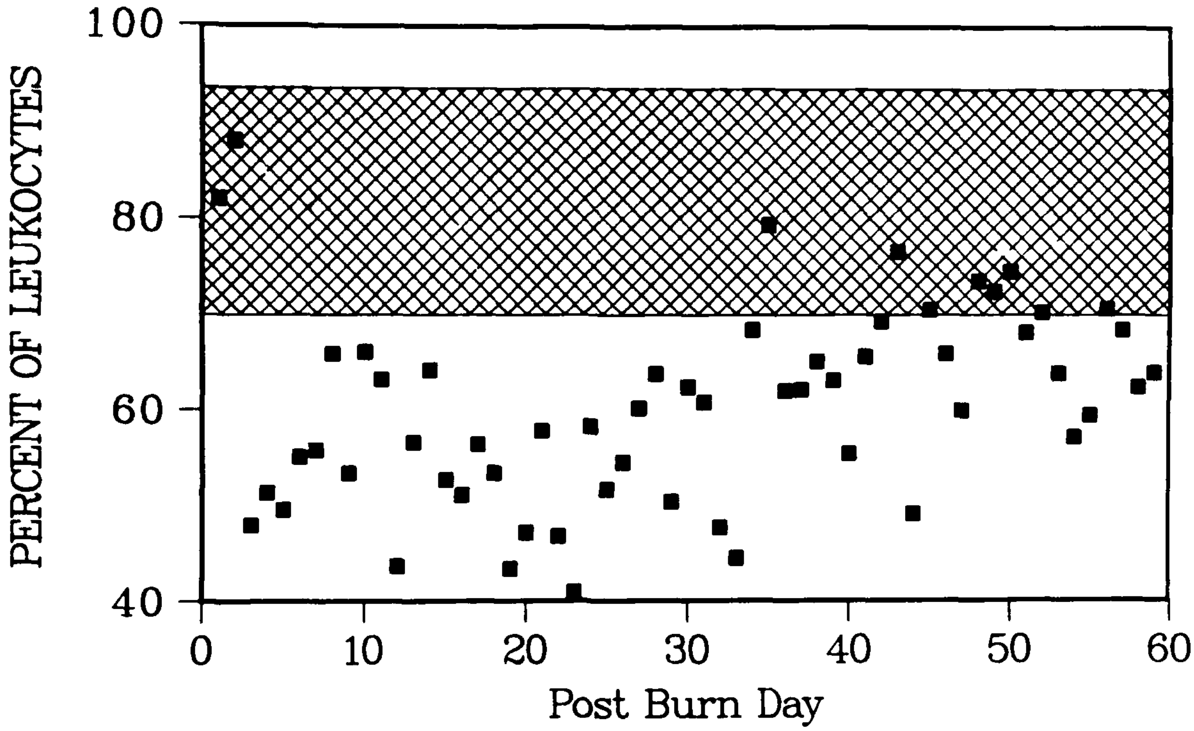

FIGURE 3. Lymphocyte counts of Ficoll-Hypaque gradient purified samples from burn patients. The mean % of lymphocytes *determined from* Wright's stain differential of a portion of the lymphocyte preparation for burn patient samples is displayed vs. postburn day. Control subject values ± 1SD are represented by the horizontal bar.

Lymphocyte subpopulations may also be compared as the absolute number of circulating cells rather than as a relative percentage. This is an estimate of the total number of cells of a particular subset available in the circulation rather than the proportion of circulating lymphocytes that the subset represents. Since burn patients were lymphopenic (Fig 2), the comparison of the number of cells present per volume of blood resulted in even wider differences in T lymphocyte subsets between burn patients and control subjects (Fig 5). In spite of the lymphopenia in burn patients, the absolute number of B lymphocytes was still increased above control.

Analysis of Other Lymphocyte Surface Markers. The proportion of HLADR positive cells (HLADR is present on some B lymphocytes, monocytes, and activated T lymphocytes) was increased in burn patients (Fig 6), but the absolute number of HLADR positive circulating cells (data not shown) did not change. The proportion of other surface antigens, Leu-7 positive cells (which includes some cytotoxic T lymphocytes and

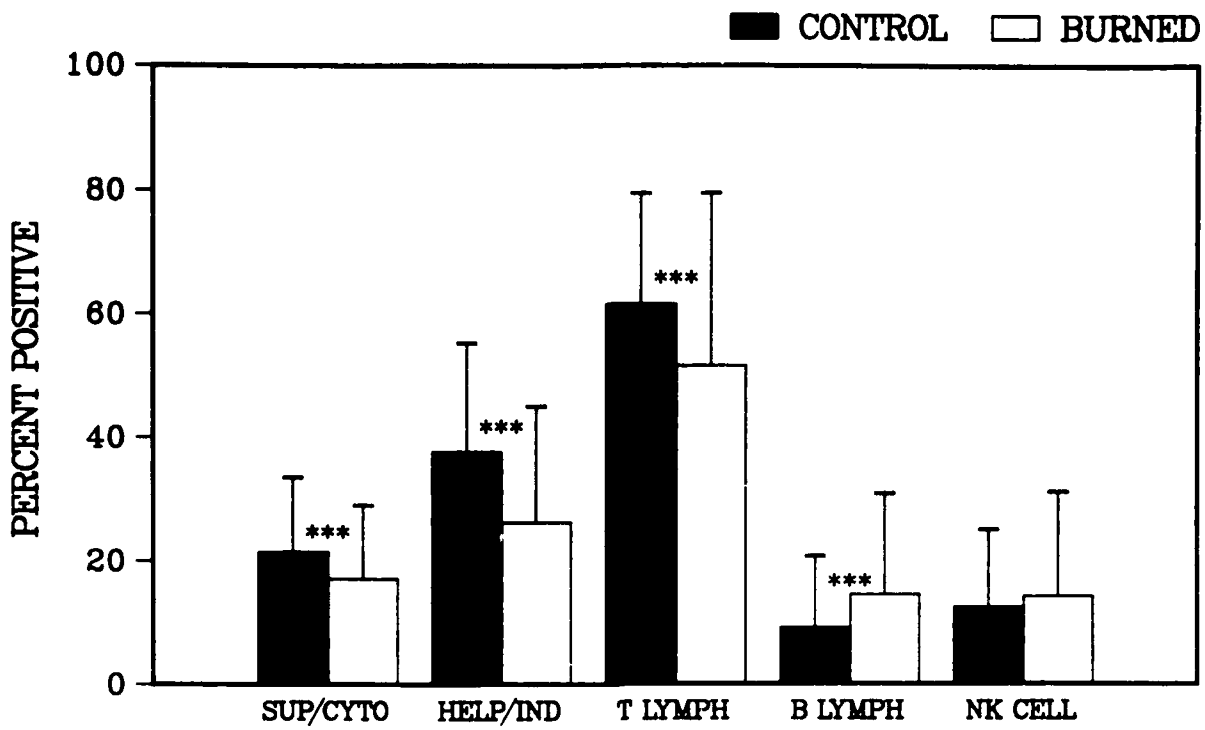

FIGURE 4. Lymphoid subpopulations of burn patients and control subjects. The mean % of each subpopulation is displayed ± 1SD for both groups. SUP/CYTO, suppressor/cytotoxic; HELP/IND, helper/inducer; T LYMPH, pan T lymphocytes; B LYMPH, B lymphocytes; NK CELL, natural killer cell. *P < 0.05, **P < 0.01, ***P < 0.001.

NK cells), Leu-8 positive cells (which includes portions of both the helper and suppressor T cell subsets), transferrin receptor positive cells (transferrin receptor is found on the surface of activated T lymphocytes and monocytes), and monocytes (Leu-M3 positive cells) (also shown in Fig 6) were not different from control subjects. With the exception of Leu-8 positive cells (which were decreased), the absolute number of cells positive for these surface markers was also not different between burn patients and control subjects (data not shown).

The helper/supressor ratio is often used as a measure of immunosuppression and has been reported by others (1,2) to be decreased in burn patients. In this study, the ratio in burn patients was identical to that of controls (Table 1). Although helper cells were decreased in these patients, the suppressor cells were proportionately decreased, and thus, the ratio remained unchanged.

TABLE 1. Helper/Suppressor Ratios ($\pm$ SD)

	N	Average	N	Average	P Value
Control vs. burn	289	2.07 ± 1.69	286	2.09 ± 1.59	NS
Survivor vs. nonsurvivor	251	1.97 ± 1.54	35	2.92 ± 1.77	= .0043

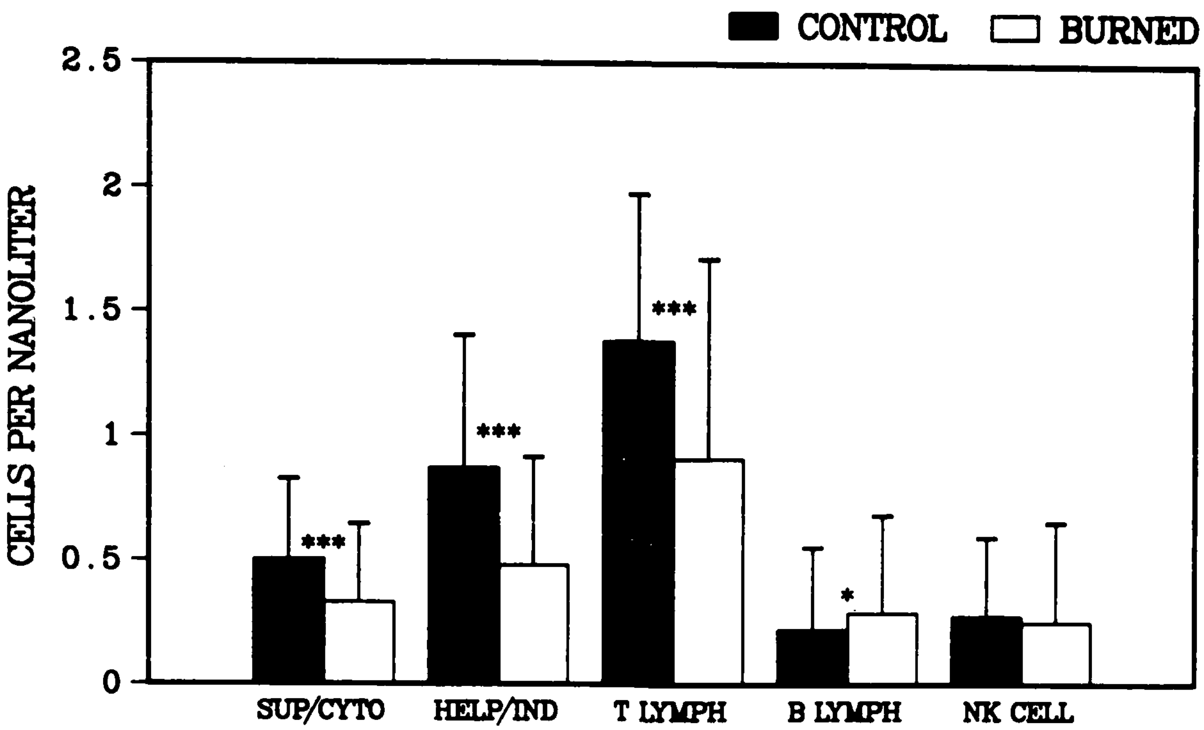

FIGURE 5. Absolute count of circulating lymphoid subpopulations of burn patients and control subjects. The *mean* % of each subpopulation is displayed $\pm$ 1SD for both groups. SUP/CYTO, suppressor/cytotoxic; HELP/IND, helper/inducer; T LYMPH, pan T lymphocytes; B LYMPH, B lymphocytes; NK CELL, natural killer cell.

Comparison of Subsets from Burn Patients Based on Mortality. Of the 33 burn patients in the study, 24 (72.6%) survived their injury. The subpopulations of survivors and nonsurvivors were compared. The proportion of the helper and suppressor T lymphocyte subsets were decreased for patients who died compared to those who lived (Fig 7). Since the proportion of helper and suppressor subsets in burn patients are smaller than that of control subjects (Fig 4), the decrease in patients who died represents a further decrease from control levels. The proportion of pan T lymphocytes and B lymphocytes was not different between the two groups. This indicates that the T lymphocytes in nonsurvivor samples were negative for both CD4 and CD8 and probably less mature than the T lymphocytes in survivors, since the CD4 and CD8 subset markers are acquired after the T lymphocyte receptor (Leu-4 or CD3) during the differentiation of T lymphocytes. The proportion of Leu-8 positive, NK cells, and transferrin positive cells were increased in patients who died, while the proportion of Leu-15 positive and large granular lymphocytes were decreased (Fig 8).

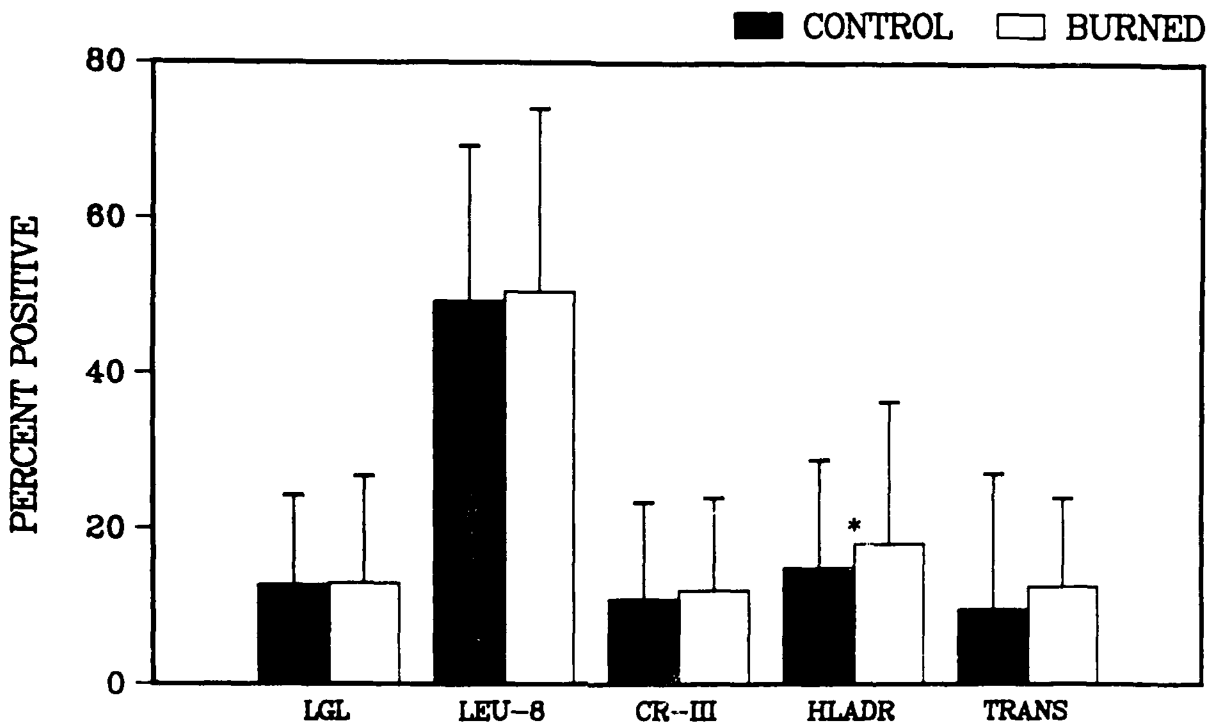

FIGURE 6. Proportions of other antigen positive populations of burn patients and control subjects. The mean % of cells positive for each of the surface antigens is displayed ± 1SD for both groups. LGL, large granular lymphocyte; LEU-8, anti-Leu-8 positive; CR-III, complement receptor 3; HLADR, HLADR positive; TRANS, transferrin receptor. $*P < 0.05$, $**P < 0.01$.

The helper/suppressor ratio (Table 1) was lower for survivors than for nonsurvivors. This reflected a greater decrease in CD8 positive cells in nonsurviving patients.

DISCUSSION

These data are consistent with the repeated observations of decreased in vivo and in vitro T cell function (6-21) in burn patients. The relationship between these observations and the decreased host resistance in burn patients is still only coincidental. No definitive mechanisms have shown how decreased T lymphocyte function leads to increased opportunistic infection in burn patients. It is possible that the decreased circulating T lymphocytes are a reflection of the hormonal and metabolic changes that occur in burn patients and not a contributing cause to this increase in infection susceptibility.

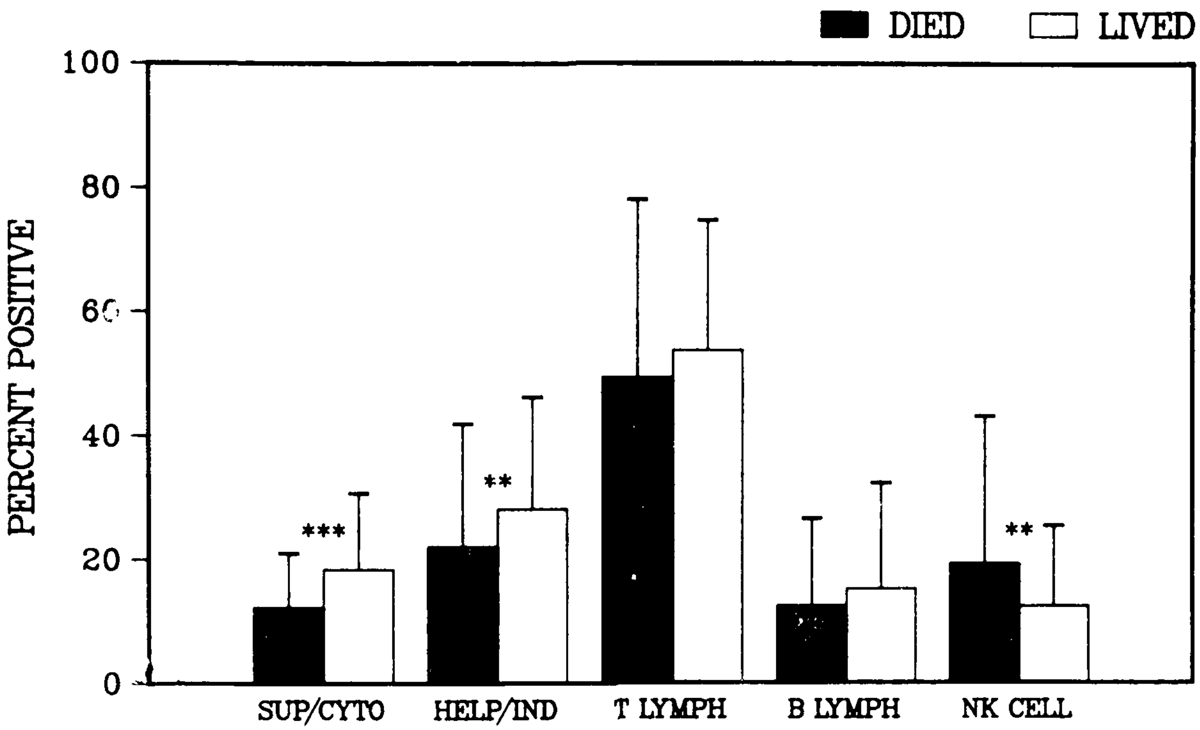

FIGURE 7. Proportions of lymphoid populations of burn patients. The mean % of each subpopulation for surviving and nonsurviving patients is displayed ± 1SD. SUP/CYTO, suppressor/cytotoxic; HELP/IND, helper/inducer; T LYMPH, pan T lymphocytes; B LYMPH, B lymphocytes; NK CELL, natural killer cell.

Our study demonstrated no difference in the helper/suppressor ratio between burn patients and control subjects. Although there was a significant difference in the ratio between surviving and nonsurviving patients, the ratio was higher in patients who died, the opposite of that in predicted if the proportion of suppressor cells contributes to immunosuppression in burn patients. The increase in the ratio in nonsurviving patients was due to a sharper decrease in CD8 positive cells in patients who died.

CD8 positive cells include the cytotoxic subset of lymphocytes which, in combination with "help" from CD4 positive cells, are the "effector" cells for delayed hypersensitivity reactions. The anergy of burn patients to these reactions and the decreased rejection of skin grafts were the first noted deficiencies of the immune response of burn patients (22-24). The extent of the anergy is related to the extent of injury (25). The sharp decrease in CD8 positive cells we observed in burn patients is consistent with this long-standing observation.

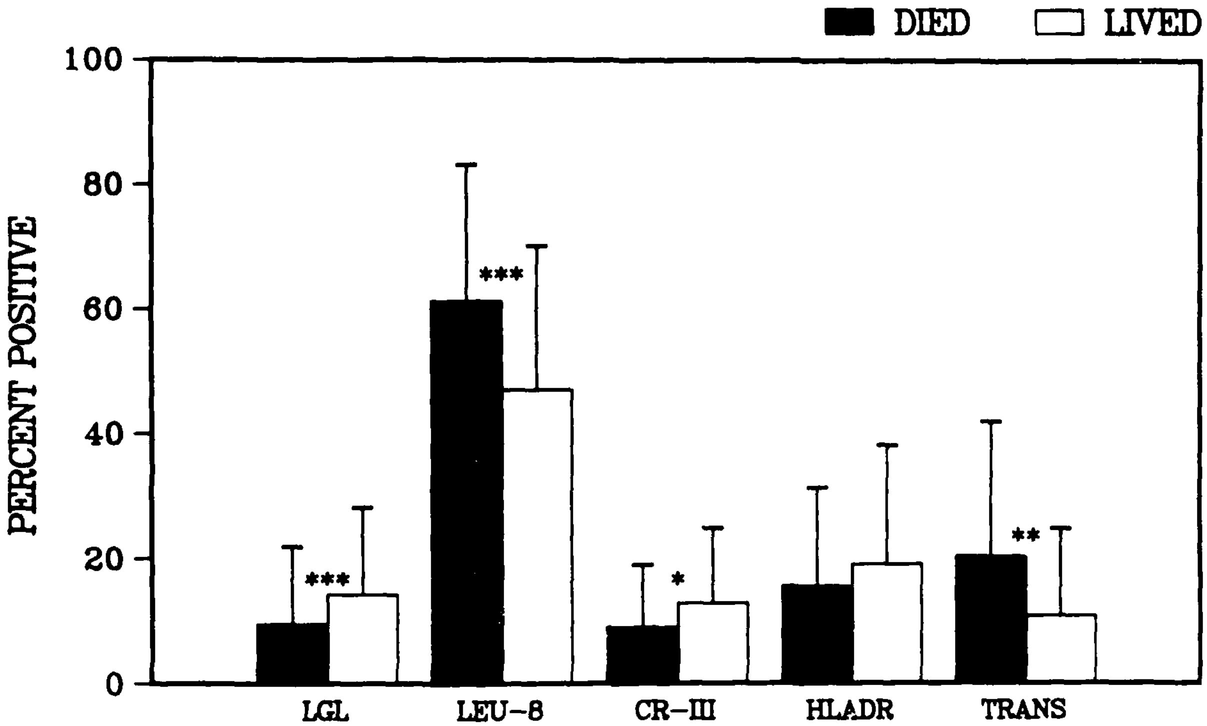

FIGURE 8. Proportion of other antigen positive populations of burn patients. The mean % of each subpopulation for *surviving and nonsurviving patients is displayed* ± 1SD. LGL, large granular lymphocyte; LEU-8, anti-Leu-8 positive; CR-III, complement receptor 3; HLADR, HLADR positive; TRANS, transferrin receptor.

The decrease in the proportion of large granular lymphocytes while NK cells increased was unexpected since NK cells are large granular lymphocytes. However, cytotoxic cells (CD8 positive) also can be large granular lymphocytes and the decrease in large granular lymphocytes may reflect the loss of the cytotoxic lymphocyte subset from the circulation of these patients. The decrease in the Leu-15 antigen (complement receptor III) may also reflect the selective loss of cytotoxic (CD8 positive) lymphocytes which carry the Leu-15 antigen.

The greatest difference between our study and previous reports is the magnitude of the decrease in CD8 positive cells we found in our burn patients. McIrvine et al (1), using fluorescence microscopy to detect bound antibody, described an increase in CD8 positive cells in burn patients. In a subsequent report from that laboratory (3) which employed flow cytometry to measure positive cells, an early decrease but no increase in CD8 positive cells was found. Antonacci et al (2) reported no change in CD8 positive cells in the first 48 h. Data were presented showing a subsequent decrease in CD8

positive cells but no comment was made concerning the statistical significance of these decreases. The proportion of CD4 positive cells in patients was decreased in all the studies. However, the differences in the decrease in CD8 positive cells meant that the ratio of CD4 to CD8 positive cells was different in all three reports.

Although the proportion of B lymphocytes in burn patients had not been reported previously, Antonacci et al (2) had reported a small but statistically insignificant increase in anti-HLADR positive cells. Anti-HLADR binds to monocytes, activated T lymphocytes, and B cells. In our hands, the proportion of HLADR positive cells increased significantly in burn patients. Since the proportion of B cells increased, the increased HLADR positive cells may be included in this population.

The reason for the differences between our results and those of previous reports is unknown. The most likely explanation is difference in technique. For example, the data of McIrvine et al (1), which were based on fluorescence microscopy, did not agree with the subsequent report based on flow cytometry (3), even though the data were from the same laboratory. The data from the later report more closely reflected those in this study. The severe lymphopenia accompanied by leukocytosis in burn patients makes isolation of granulocyte-free lymphocyte preparations on standard Ficoll-Hypaque gradients impossible. As contamination increases, it is increasingly difficult to exclude nonlymphoid cells from analysis by electronic gating on light scatter. Contaminating cells can alter the final data in two ways. If they bind staining reagents nonspecifically, which is frequently the case, they may increase the percent of positives determined. If they do not, they may decrease the proportion of positives by increasing the number of negatives. In these contaminated preparations, information on the isotypic controls used, how the positive cutoff was determined, or how the lymphocyte gates were set are critical if accurate comparisons are to be made. Unfortunately, these parameters were undisclosed or only partially disclosed in the previous reports.

To obtain consistently accurate results, we have found it necessary to match isotypic controls conjugated to marker chromophores to that of the monoclonal reagents being used. Additionally, we monitor nonlymphoid cell contamination in all analyses with a reagent (anti-Leu-M3) that binds most nonlymphoid leukocytes but does not bind lymphocytes. Without more accurate information on the techniques used in previous reports, it is not possible to directly compare the data.

PRESENTATIONS

Burleson DG: Measurement of peripheral blood lymphocyte subpopulations in burned patients. Presented at the 11th RES Congress and 24th National Reticuloendothelial Society, Kuaui, Hawaii, 20 October 1987.

Burleson DG: Measurement of in vitro function of B lymphocytes from burned patients. Presented at the 20th Annual Meeting of the American Burn Association, Seattle, Washington, 24 March 1988.

Burleson DG: The effect of intravenous immune globulin administration on lymphocyte phenotype and function in burned patients. Presented at the 8th Annual Meeting of the Surgical Infection Society, San Francisco, California, 5 May 1988.

PUBLICATIONS

Burleson DG, Mason AD Jr, and Pruitt BA Jr: Lymphoid subpopulation changes after thermal injury and thermal injury with infection in an experimental model. Ann Surg 207(2):208-12, February 1988.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA311489	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	K	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	077
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) A Study of Biochemical Changes in the Cellular Environment of Tissue of the in vivo Partial-Thickness Rat Burn Wound

12. SUBJECT AREAS
06 01 Biochemistry 06 04 Anatomy and Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 09	88 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Basil A. Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.0	30
89	0.0	0

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BROWN, W L
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4652
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): MASON, A D
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn Wound Metabolism; (U) Edema; (U) Burn Injury; (U) Lab Animals: (U) Rats; (U) ILIR; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Determination of the biochemical and metabolic changes that occur in the in vivo partial-thickness rat burn wound during the early postburn period and identification of criteria of reversibility. The data generated may identify means to block or reverse the metabolic changes and limit the progression in the extent and severity of injury in wounds of burned soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) Microelectrodes will be used to measure changes in extracellular potassium ion content and in pH and/or carbon dioxide partial pressure at various sites in the in vivo burn wound. Samples from sites adjacent to the microelectrodes will be taken to measure selected metabolites using enzymatic methods. Cells and subcellular organelles will be isolated for measurement of changes in function with time postburn.

25. (U) 8710 - 8809. We have completed the series of measurements of water content, dry weight, and pH of 20% sham and partial-thickness scald burn wounds from 1-72 h postburn. We are currently testing methods for separating epidermal and dermal cells to be used in studies of the effect of tissue pH and time postinjury on cell function. This project was transferred to DA315351.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: A Study of Biochemical Changes in the Cellular Environment of Tissue of the *in vivo* Partial-Thickness Rat Burn Wound

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Wanda L. Brown, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr, MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: A Study of Biochemical Changes in the Cellular Environment of Tissue of the *in vivo* Partial-Thickness Rat Burn Wound

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: Wanda L. Brown, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Partial-thickness burn wounds contain cells that have undergone potentially reversible injury. Massive postburn edema, however, by disturbing blood flow and capillary density, interferes with gas exchange, nutrient delivery, and removal of metabolic end products and may create an environment hostile to cellular recovery. Similarly, agents released locally as components of the inflammatory process may interfere with cellular restitution, and the presence of such agents may interact with impaired blood flow to deter recovery further. Conversely, some of these changes may have favorable influences. Flow-driven limitations of metabolic activity might, in a sense, permit the injured cell to rest and recover. The purpose of this study is to assess the roles of postinjury changes in partial-thickness burn wounds in either fostering or impeding cellular recovery.

A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE IN VIVO PARTIAL-THICKNESS RAT BURN WOUND

In a previous report (1), we showed that the quantity and time course of edema accumulation in partial- and full-thickness burn wounds in rats were similar during the first postinjury day. Beyond 24 h, however, edema volume decreased more rapidly in partial-thickness injuries. In both kinds of injury, burn wound tissue pH decreased during the period of maximal edema, suggesting accumulation of acid metabolites.

MATERIALS AND METHODS

Young Sprague-Dawley rats weighing 180-200 g were anesthetized with alpha-chloralose (5.5 mg/100 g IP). The hair on the dorsum was clipped and the rats were placed in a protective mold which limited the area to be burned to 20% of the total body surface area. Immersion of the exposed area in water at 80°C for 8 sec produced a partial-thickness burn. Sham controls were anesthetized, clipped, placed in the protective mold, and an equivalent area on the dorsum was outlined in ink. The rats were housed in individual cages and permitted free access to food and water. No parenteral fluids were administered. At selected times postburn, the rats were reanesthetized with alpha-chloralose for insertion of electrodes or excision of wound samples. The rats were then sacrificed by injecting an overdose of alpha-chloralose without having been allowed to reawaken.

Tissue Sampling Procedures. Wound areas were excised to underlying fascia. For determination of water content and dry weight of the wounds, the entire sample, including the panniculus carnosum, was weighed immediately and then dried to constant weight.

In cases where the wound tissue was to be used for isolation of cells, the sample was removed as described above, quickly chilled, and the panniculus carnosum and fatty layer were removed by dissection and by scraping the lower surface with a dull scalpel. The upper surface of the wound tissue was wiped with a gauze sponge soaked in acetone in order to remove surface lipids and then rinsed 3X in ice-cold isotonic saline, blotted, cut into halves, weighed, and kept in a dish placed on a bed of crushed ice while being cut into pieces. In some cases (see RESULTS), each half was either used intact, scored through the dermis in approximate 4-mm squares with the epidermal surface intact, cut into approximate 4 X 8-mm slices, or cut into 3- to 4-mm square pieces (diced).

Enzyme Solutions.

I. Prepared in Hepes buffered balanced salt solution containing Ca++, no Mg++, and supplemented with 10 mM glucose, pH 7.4 (2).

A. Trypsin from bovine pancreas (Worthington Biochemical, Freehold, NJ), 193 U/ml.

B. Collagenase CLS 4 from *Clostridium histolyticum* (Worthington Biochemical), 204 U/ml, and hyaluronidase (Wyeth Laboratories, Philladelphia, PA), 5.6 U/ml.

II. Prepared in Dulbecco's phosphate-buffered saline without Ca++, Mg++, or glucose, pH 7.4.

A. Dispase, Grade II from *Bacillus polymyxa* (Beohringer Mannheim Biochemicals, Indianapolis, IN), 0.8 U/ml (3).

B. Collagenase from *Achromobacter iophagas* and diphase, Grade II from *Bacillus polymyxa* mixture (Beohringer Mannheim Biochemicals), 0.1 U/ml and 0.8 U/ml, respectively (4).

Ten milliliters of the selected enzyme solution were added to each tube containing the tissue from one-half of the wound. The tubes were capped and the mixture vortexed and incubated at 37°C in a shaking water bath at 180 oscillations/min (see DISCUSSION). After that time, the contents of the tubes were mixed by vortexing and were chilled by placing the tubes in a container of crushed ice. The chilled preparation was filtered through nylon monofilament screening fabric (Tetko, Inc., Elmsford, NY), with 70- or 112-μ openings, into plastic tubes. The tissue retained by the filter was washed 2X with 1 ml buffer and the filtrate was centrifuged at 4°C for 10 min at 800 g. The supernate was decanted and the cell pellet was resuspended in 5 ml buffer, centrifuged, and decanted 2X. The cell pellet was then resuspended in 2 ml buffer and maintained at 4°C.

The cells in the samples were counted using a hemocytometer. Viable and nonviable cells were differentiated using a trypan-blue procedure (5). Representative samples, both untreated and enzyme-treated, were fixed in formalin, sectioned, and stained with H&E for histological examination.

RESULTS/DISCUSSION

In our previous report, we showed that the 20% partial-thickness rat burn wound contained a mean of 3.6 ml more water at 1 h postburn (PB) and 4.6 ml more water at 3 h PB

than the equivalent sham wound. The quantity of excess water in the burn wound increased to a maximum of 7.8 ml at 18 h PB, after which it decressed slowly to 3.6 ml excess at 72 h PB. Subsequent measurements have shown that in those wounds in which the surface remains intact, the partial-thickness burn wound still contains approximately 1.5 ml excess water at 7 days PB. Burn wounds in which the surface was abraded contained less water than sham wounds at 7 days PB.

Tissue incubation for 45 min in tryspin solution in Hepes buffer (see para IA) released only a very small number of cells into the solution from samples taken at 1, 24, 48, and 72 h PB. The hair and epidermis of the burn wound could be separated from the dermis by scraping with a sharp knife but remained attached when the sham wound was scraped. This was confirmed histologically and is in contrast to results reported for human, porcine, and neonatal rat skin (6). When this same tissue (unscraped or scraped) was washed with buffer and then incubated for 105 min in collagenase/hyaluronidase solution (see para IB), a much larger yield of individual cells was obtained which were impermeable to trypan-blue and considered to be viable. The preparations were contaminated with both erythrocytes and with numbers of neutrophils that varied to some extent with time postburn.

Tissue samples incubated in collagenase/hyaluronidase in Hepes buffer (see para IB) for 150 min produced a quantity and type of cells not greatly different from those samples incubated first in trypsin as described above. In every case, the yield of cells was greater when the tissue was cut into 2- to 4-mm pieces.

Dispase has been reported to be the enzyme of choice for separating the epidermis from the dermis of human skin for use in cell cultures (5). However, for neither sham or burn wound tissue did incubation in dispase alone (see para IIA) or in a mixture of dispase and collagenase from Achromobacter iophagus for an hour result in changes in the tissue that led to easy separation of the two layers by dissection. The two layers of both sham and burn wounds could be separated only by scraping the epidermis and hair off with a sharp blade. Few free cells were released into the solution, compared to those released by incubation with collagenase/hyaluronidase. Dispase also destroyed the staining characteristics of the dermis. Although we used phosphate-buffered saline to dissolve the dispase enzymes as recommended by the suppliers, we showed that the pH falls below 7 during the incubation period, whereas the solutions prepared in Hepes buffer maintained a pH of 7.4 during incubations lasting as long as 180 min. Based on these results, we conclude that dispase and the mixture of dispase/collagease are not suitable for isolating cells from adult rat skin.

We have subjected a few of the cell preparations to isopyknic Percoll gradient density centrifugation at several differant gravity forces and lengths of time in an attempt to separate the different types of skin cells from the containing cells with some success. More tests will be required to determine the ideal conditions for producing bands of pure cell types to be used in proposed studies of cellular function. We are also currently testing procedures for sampling with instant freezing of biopsy samples and various methods for determining the content of selected metabolites in sham and partial-thickness rat burn wounds.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA313322	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	K	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	078
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) The Effect of Interleukin-2 Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

12. SUBJECT AREAS
06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 10	88 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY [signature]
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE a. FISCAL YEARS	PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.5	30
89	0.0	0

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR CIOFFI, W G
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-4440
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Interleukin-2; (U) Pseudomonas; (U) Sepsis; (U) Burns; (U) Lab Animals: (U) Rats; (U) ILIR; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The effects of exogenous interleukin-2 administration following burn wound infection will be studied in a rodent model. A literature search was performed and indicated no duplication of effort.

24. (U) Two iterations of the study were performed to determine the optimal dose of interleukin 2 and the optimal postburn day for administration. Upon completion of these studies, two addenda were submitted and approved. The project will be continued with the addition of indomethacin in high and low doses administered concomitant with interleukin 2 in an attempt to increase interleukin 2 receptor expression.

25. (U) 8710 - 8809. The optimal dose which could be tolerated by the septic animal was identified. In LD100 and LD50 models, interleukin 2 administration concomitant with burning and seeding of the burn wound with Pseudomonas failed to result in improved survival. To increase interleukin 2 receptor expression, both high doses (5 mg/kg) and low doses (0.5 mg/kg) of indomethacin were administered with the interleukin 2. This failed to improved survival in the LD100 model. This project was transferred to DA315353.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE * USGPO 1986-491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of Interleukin 2 Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Theresa A. Graves, MD, Captain, MC
Carlin V. Okerberg, DVM, PhD, Major, VC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of Interleukin 2 Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Theresa A. Graves, MD, Captain, MC
Carlin V. Okerberg, DVM, PhD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Decreases in interleukin 2 (IL 2) production, activity, and receptor expression have been described following injury. Previous investigators have reported increased survival following pretreatment with recombinant IL 2 in secondary infection, thermal injury models. We have studied the effects of exogenous recombinant IL 2 administration on survival in a rat model of Pseudomonas burn wound sepsis. Sprague-Dawley rats received 20% full-thickness total body surface burns. These wounds were then inoculated with Pseudomonas aeruginosa (Strain 1244, LD100 model) immediately following thermal injury on postburn day 2. Groups of infected rats then received varying doses of recombinant IL 2 twice daily for a 7-day period prior to, concomitant with, or 3 days following inoculation. Control groups received the burn, inoculation, and IL 2 matrix only. Recombinant IL 2 doses ranged from 1.5×10^5 to 6×10^7 U/kg. Autopsies were performed on all animals and the presence of microabscesses in the lung and liver were documented. Recombinant IL 2 administration failed to prolong survival or increase the survival rate of any group. The rates of distant microabscesses in the lung and liver were not altered. In an attempt to increase IL 2 receptor expression, several groups of IL 2-treated rats also received indomethacin once daily. Hoever, the addition of indomethacin failed to increase survival. We conclude that recombinant IL 2 therapy failed to improve survival in this model of invasive burn wound sepsis.

THE EFFECT OF INTERLEUKIN 2 ADMINISTRATION ON MORTALITY TO RATS WITH PSEUDOMONAS BURN WOUND SEPSIS

Interleukin 2 (IL 2) has been administered to animals and humans with nonthermal injuries in attempts to improve cell-mediated immunity (1-2). IL 2 production, activity, and receptor expression have been measured in animals and humans following thermal injury. The effects of exogenous IL 2 administration following thermal injury on survival has not been studied.

Following a severe thermal injury, alterations in the host immune system develop, which lead to depression of the immune response (3-5). Defects in cellular and humoral systems have been reported. These defects are manifested by increased susceptibility to sepsis, impaired delayed hypersensitivity reaction, and prolongation of allograft rejection.

The defect in the cell-mediated immune system is thought to be secondary to impairment of T lymphocyte activity, with a decrease in the overall number of circulating T cells postburn. These levels return to normal by postburn day 40 in surviving patients. In nonsurvivors, the T cell population does not fully regenerate. The OKT_4 helper/inducer ratio also decreases immediately postburn (6) and later returns to normal in survivors.

It has been suggested that the decrease in the helper to suppressor cell ratio is an effect of the infectious process that occurs after a burn but does not necessarily correlate with the infection susceptibility of a burn injury model (7).

As the mechanisms for T cell interactions become clearer, it is apparent that IL 2 (8) is produced by T helper cells. The stimulus for this IL 2 product by T helper comes from circulating interleukin 1, which is itself released from macrophages when they are exposed to foreign antigens (9). The IL 2 then binds to specific IL 2 receptors to promote the proliferation of T lymphocytes, regardless of their antigenic specificity (10). The number of IL 2 receptors, the amount of IL 2 circulating, and the length of contact between the two all seem to be important in the magnitude of the T cell response.

Assays of interleukin 1 produced in man in response to thermal injury have been measured and found to be increased immediately postburn and then subsequently return to normal (9).

IL 2 production has been shown to be significantly reduced postburn and only returns to normal in those patients who eventually survive (9). IL 2 receptors are also decreased on the T cells following thermal injury (6). This has been

related to the ability of IL 2 to modulate the expression of its own receptor.

The addition of exogenous IL 2 to the sera of immunosuppressed burn patients leads to an increase in the percentage of IL 2 receptor expressing cells in vitro (11). In one study by Antonacci et al (12), this increased the responsiveness of AMLR culture to further stimulation. However, Teodorczyk-Injeyan et al (6) showed that the increase in IL 2 receptor cells was not proportional to the ability of the cultures to proliferate in response to antigenic stimulation. This lead to the suggestion that there are low and high affinity receptors and that this failure to respond to stimulation is the result of proliferation of low affinity receptor pools. A functional defect is thus maintained, which is most pronounced in nonsurviving burn patients.

The appropriate dose of IL 2 in the rat has not been well defined. In the murine models, both the route and dosing interval appear to be important in obtaining a maximal response. The peak levels of IL 2 are greatest when it is given intravenously, but this results in a short serum half-life (13). When the drug is administered either subcutaneously or intraperitoneally, the peak levels are lower but the half-life is prolonged. Toxicity is also related to peak serum concentration. A dosing schedule of smaller injections given more frequently should be optimal (14). Human subjects tolerate much smaller doses of IL 2 than the rat and appear to sustain increased morbidity from intravenous injections. Pulmonary edema and compromise are the most common complications. In the rats, a dose of 10^6 U/kg over 48 h was well tolerated without toxicity. The most common adverse affect in rats is liver toxicity.

IL 2 is available from human lymphocyte-conditioned media or as human recombinant IL 2. The molecular weight and activity per unit appear to be comparable for both forms (8).

The role of IL 2 in the immune response following thermal injury remains unclear. There is a definite temporary decrease in the T cell population which is proportional to the decrease in cell-mediated immune response. Co-incident with this is an increase in interleukin 1 production from the monocyte-macrophage cell lines. This does not lead to the expected increase in IL 2 production that is found in normals. The amount of IL 2 appears significantly depressed and remains so despite the gradual normalization of other immune cell pools (15). Whether the decrease in the circulating amount of IL 2 is an important regulator of the T cell system or whether the ability of IL 2 to stimulate only the production of low affinity receptors needs to be investigated further.

Whether the addition of exogenous IL 2 to burn patients can upregulate the production of IL 2 receptors enough to promote an increase in T cell function and thus improve the T cell-mediated response is unknown. We propose to study the effect of IL 2 on survival in an infected burn rat model.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing approximately 200 g were used throughout the study. The Pseudomonas burn wound sepsis model as first described by Teplitz (16-18) was used. All rats received a 20% total body surface full-thickness scald burn (19). Animals in the infection groups had their wounds inoculated with 1 cc of 1 X 10^7 *Pseudomonas aeruginosa* (Strain 1244) on postburn day 2. This model reliably results in severe burn wound sepsis (LD100). These groups of inoculated rats received varying doses of recombinant IL 2 intraperitoneally in 1 ml of 5% dextrose twice daily for a 7-day period. IL 2 administration began 2 days prior to, concomitant with, or 3 days following inoculation of the burn wound with *Pseudomonas aeruginosa*. Animals in the control groups received the burn, infection, and IL 2 matrix or saline only. The IL 2 doses ranged from 1.5 U/kg (0.5 mg/kg to 6 X 10^7 U/kg) to 2 mg/kg. Necropsies were performed on all animals to document any microabscesses in the lung and liver. Survival was followed for 20 days, at which time, any survivors were euthanized and necropsied.

In an attempt to increase IL 2 receptor expression by decreasing prostaglandin E_2 levels, several groups of IL 2-treated rats also received indomethacin. *In vitro* work has also suggested that prostaglandin synthesis inhibition increases IL 2 receptor expression. Rats received either 0.5 or 5.0 mg/kg indomethacin daily with IL 2 administration.

RESULTS

Phase I. Six groups of 10 rats each received 20% total body surface area full-thickness scald burns. The wounds were inoculated with *Pseudomonas aeruginosa* on postburn day 3. Group A animals received the IL 2 matrix. Group B animals were administered 2 mg/kg IL 2, Group D was administered 0.2 mg/kg IL 2, Group E was administered 0.1 mg/kg IL 2, and Group F was administered 0.05 mg/kg IL 2. Treatments were administered twice daily for 7 days. Results are shown in Table 1. Groups B and C (the higher IL 2 doses) demonstrated a decreased survival as compared to the Group A (control group). There was no difference in mean survival time for Groups D, E, and F as compared to Group A. The incidence of distant microabscesses was the same for all groups except Group B. In this group, the rate of abscess formation in the lungs was only 10% (1 out of 10 animals).

TABLE 1. Results for Phase I

Group	n	Treatment	Survival (Days)*	Abscess Formation (%) Lung	Liver
A	10	Matrix	11.4	80	30
B	10	2 mg/kg	6.2	10	10
C	10	1 mg/kg	7.8	60	20
D	10	0.2 mg/kg	9.7	70	20
E	10	0.1 mg/kg	11.8	70	50
F	10	0.05 mg/kg	11.1	90	10

*100% mortality for all groups.

Phase II. Five groups of 20 rats each received 20% total body surface area burns. The wounds were inoculated with <u>Pseudomonas aeruginosa</u> on postburn day 2. IL 2 was administered on a twice daily basis beginning on postburn day 0 for 7 days. Group A animals received saline only, Group B received the IL 2 matrix only, Group C received 0.2 mg/kg IL 2, Group D received 0.1 mg/kg IL 2, and Group E received 0.05 mg/kg IL 2. Pretreatment with IL 2 failed to prolong survival of any group as compared to the two control groups (Table II). The rates of microabscess formation in the lung and liver were similar for all groups.

TABLE 2. Results for Phase II

Group	n	Treatment	Survival (Days)*	Abscess Formation (%) Lung	Liver
A	20	Saline	16.8	70	10
B	20	Matrix	16.4	80	5
C	20	0.2 mg/kg	15.3	70	10
D	20	0.1 mg/kg	15.3	70	20
E	20	0.05 mg/kg	15.5	95	20

*100% mortality for all groups.

Phase III. Low-dose indomethacin (0.5 mg/kg) was administered to several groups in an attempt to increase IL 2 receptor expression. Five groups of 20 rats received 20% total body surface area burns and the wounds were inoculated with *Pseudomonas aeruginosa* on postburn day 0. IL 2 and indomethacin administration began on postburn day 0 and continued for 7 days. Group A received saline only, Group B received 0.2 mg/kg IL 2 twice daily, Group C received 0.5 mg/kg indomethacin once daily, Group D received both IL 2 and indomethacin, and Group E received IL 2 but the burn wound was not inoculated. Addition of indomethacin failed to increase the survival of any group. The rates of distant microabscess formation were not different between any groups. Group E demonstrated no toxicity to IL 2, with all but one animal surviving for 20 days. No abscess formations were noted.

TABLE 3. Results for Phase III

Group	n	Treatment	Survival (Days)	Abscess Formation (%) Lung	Abscess Formation (%) Liver
A	20	Saline	9.8*	70	75
B	20	Interleukin 2	10.6*	60	30
C	20	Indomethacin	10.3*	40	15
D	20	Interleukin 2/ Indomethacin	9.7*	65	15
E**	20	Interleukin 2	19.5	0	0

*100% mortality.
**This group was not inoculated with *Pseudomonas aeruginosa*.

Phase IV. For this phase, high-dose indomethacin (5 mg/kg) was administered. Four groups of 20 rats received 20% total body surface area burns and the wounds were inoculated with *Pseudomonas aeruginosa* on postburn day 0. IL 2 and indomethacin administration began on postburn day 0 and continued for 7 days. Group A received saline only, Group B received IL 2 (0.2 mg/kg) twice daily, Group C received indomethacin (5 mg/kg) once daily, and Group D received both IL 2 and indomethacin. The administration of IL 2 and indomethacin failed to increase mean survival time (Table 4).

DISCUSSION

Previous investigators have reported increased survival following pretreatment with recombinant IL 2 in secondary

TABLE 4. Results from Phase IV

Group	n	Treatment	Survival (Days)*
A	20	Saline	7.0
B	20	Interleukin 2	6.4
C	20	Indomethacin	5.5
D	20	Interleukin 2/Indomethacin	4.6

*100% mortality for all groups.

infection thermal models. Gough et al (20) treated mice with recombinant IL 2 for 7 days following thermal injury. On postburn day 10, cecal ligation and puncture were performed. Animals receiving saline only showed 100% mortality on postburn day 14 while those receiving IL 2 had only a 55% mortality. The splenocytes harvested from IL 2-treated mice showed improved responses to T cell mitogens in vitro as compared to saline controls. In contrast to these findings, we have failed to show any benefit of exogenous IL 2 administration in our model of invasive burn wound sepsis. Interperitoneal IL 2 administration is known to cause as influx of granulocytes into the peritoneal cavity. It may well be that the increased survival seen in the cecal ligation and puncture model is secondary to the influx of granulocytes.

The failure of exogenous IL 2 to increase survival in this model of infected burn wound sepsis may have multiple etiologies. As suggested by Teodorczyk-Injeyan et al (11), exogenous IL 2 may only result in increased expression of low-affinity IL 2 receptors which does not result in an increased T cell response to antigenic stimulation. Thus, a functional defect is maintained in the milieu of increased IL 2 levels. Various manipulations have been proposed as methods to increase high affinity IL 2 receptor expression. Increased prostaglandin E_2 levels following thermal injury have been hypothesized as one mechanism which decreases IL 2 receptor expression. In this group of studies, we attempted to inhibit prostaglandin E_2 synthesis by the administration of exogenous indomethacin. Doses of indomethacin used were those reported in the literature which resulted in a decreased prostaglandin E_2 production. The administration of indomethacin concomitant with IL 2 failed to improve survival.

Future work will attempt to determine if indomethacin increases IL 2 receptor expression in vivo. Groups of rats will be administered a burn injury and indomethacin or saline. The expression of IL 2 receptors on T cells from the spleen and

peripheral blood will be assayed. Administration of exogenous gamma interferon has been suggested as another method of increasing IL 2 receptor expression. Other work will use this cytokine with IL 2.

Finally, it may be that this infection model is so severe that improvements in T cell function will not result in improved survival. Infection of dead, devitalized tissue does not lend itself to inhibition of microbial growth by alterations in immune function. The ability of these and other cytokines will be investigated as methods to decrease morbidity and mortality in other infection models (i.e., pulmonary sepsis following thermal injury).

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA314559	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
88 09 16	K	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	079
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Development of Thermal Ionization Mass Spectrometry Methodology for the Study of Calcium Metabolism

12. SUBJECT AREAS
06 01 Biochemistry 06 04 Anatomy and Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
88 05	88 09	DA	C

MILITARY RELEVANCY CERTIFIED
APPROVED BY Basil A. Pruitt

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	0.3	20
c. TYPE	d. AMOUNT	89	0.0	0
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR SHIPPEE, R L
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-7138
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) VAUGHAN, G M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) OKERBERG, C V

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns; (U) Mass Spectrometry; (U) Metabolism; (U) Homeostasis; (U) Lab Animals; (U) Rats; (U) ILIR

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The purpose of this study is to develop thermal ionization mass spectrometry techniques to investigate the homeostatic mechanisms of calcium regulation after thermal injury. Methodology developed from this study will be applied to clinical studies of calcium metabolism in thermally injured soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) Male Sprague-Dawley rats will be used as a burn model to study the effect of burn injury on calcium homeostasis. Stable isotopes of calcium will be used to perform calcium kinetic analyses after burn injury. Total fecal and urine excretion will be collected to perform calcium balance calculations. The combined data from the kinetic analyses and the balance calculations will be analyzed using a mathematical modeling computer program to determine the effect of burn injury on the various aspects of calcium metabolism.

25. (U) 8805 - 8809. This project was approved by the USAISR Research Council and the US Army Institute of Surgical Research Animal Care and Use Committee during April 1988. Equipment and supplies have been ordered and work will be initiated upon their arrival. This project was transferred to DA315354.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * USGPO 1986 -491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA311491	2. DATE OF SUMMARY 88 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	K	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	081
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Evaluation of in vitro Cultivated Keratinocytes as Epithelial Autografts for the Closure of Burn Wounds

12. SUBJECT AREAS
06 05 Medicine and Medical Research 06 13 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	88 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	c. FUNDS (In thousands)
88	1.5	100
89	0.0	0

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BUESCHER, T M
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): CIOFFI, W G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available): MASON, A D

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Healing; (U) Keratinocytes; (U) Cell Cultures; (U) Skin; (U) Skin Graft; (U) Frozen Skin; (U) Volunteers:

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Adults; (U) RAII

23. (U) To evaluate cultured keratinocytes as grafts for epithelial closure of burn wounds. To identify technical and immunological requirements to establish banks of frozen histocompatible keratinocytes for wound coverage in burned soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) The possible utility of cultured keratinocytes will be established initially with cultured autologous keratinocytes. Keratinocytes are cultured from biopsies taken early after admission of patients with large burns and limited unburned donor sites for standard partial thickness autografts. If such grafts are deemed clinically useful, efforts will expand into investigations of allogeneic skin cultures.

25. (U) 8710 - 8809. Two patients were enrolled in the study during this reporting period. This project was transferred to DA315359.

DD FORM 1498 83 MAR   EDITION OF MAR 68 IS OBSOLETE.   * U.S.G.P.O. 1986-491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: Evaluation of in vitro Cultivated Keratinocytes as Epithelial Autografts for the Closure of Burn Wounds

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

Judson C. Lively, MD, Major, MC
Albert T. McManus, PhD
Mark R. Pittelkow, MD*
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

*Department of Dermatology
Mayo Clinic
Rochester, Minnesota 55903

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: Evaluation of in vitro Cultivated Keratinocytes as Epithelial Autografts for the Closure of Burn Wounds

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: Judson C. Lively, MD, Major, MC
Albert T. McManus, PhD
Mark R. Pittelkow, MD
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

This study evaluates the suitability of cultured autologous epithelium for the closure of burn wounds. It was designed as a pilot study consisting of 10 patients for whom cultured grafts will be grown at the Mayo Clinic. The first and only patient to date was entered into the study on 8 February 1988. Over the ensuing 3-1/2 weeks, the cultured keratinocyte grafts were successfully grown in vitro. However, they failed to survive following application to the patient's wounds. Therefore, the patient was grafted using conventional methods. The wounds healed and the patient was later discharged. No adverse reactions or side effects were noted. This study will be continued until a total of 10 patients have been enrolled.

EVALUATION OF *in vitro* CULTIVATED KERATINOCYTES AS EPITHELIAL AUTOGRAFTS FOR THE CLOSURE OF BURN WOUNDS

The extent of the skin loss caused by a burn is the major determinant of outcome. Several techniques have been developed to effect closure of the burn wound. These include autografting as well as temporary coverage with allograft skin, heterograft skin, or artificial skin substitutes. The goal of burn wound care is to achieve timely permanent closure of the open wound. At this time, the only adequate permanent cover is autograft, since all other biological membranes are temporary and artificial skin substitutes require eventual grafting with the patient's own epidermis. When the surface area and the depth of the burn are so extensive that the patient's available donor sites are insufficient to provide skin grafts to cover the wound in a timely fashion, a new source of autograft is desirable. Human keratinocytes can be cultured to produce confluent epithelial sheets. These cells can be grown from a relatively small initial sample of the patient's unburned epidermis and can be expanded, over a period of weeks, to a size sufficient to cover the entire body surface area. The use of cultured autologous epithelium in burn patients has been reported by a few institutions (1-3). It has been employed as an adjunct to conventional therapy and has resulted in successful closure of massive burns in small numbers of patients who might have otherwise succumbed to their injury. Physicians working at the Mayo Clinic (Rochester MN) have recently reported successful use of cultured autologous epithelial grafts in a patient who sustained a 99% total body surface area burn (4). This is the first report of the use of cultured keratinocytes grown in a defined medium without the use of a feeder layer of lethally irradiated mouse 3T3 cells in the culture flasks (5).

MATERIALS AND METHODS

Study Design. The objective of this study is to determine the suitability of cultured autologous epithelium for the closure of burn wounds. Initial efforts are being directed toward successfully growing transported keratinocytes and then transporting the culture-grown epithelial sheets by air transportation. This transportation system employs commercial airlines and results in approximately a 24-h delay prior to application of the cultured grafts onto the recipient's wound beds. The successful closure of the burn wounds grafted with these cultured keratinocyte grafts are then be studied. Wound coverage is compared with similar wounds covered with fresh autograft. Additional efforts will be made to determine the best recipient bed upon which to apply the cultured keratinocyte grafts and the best dressing with which to cover and protect these fragile grafts.

Number of Patients. Ten patients will be enrolled in this study. Properly signed and witnessed volunteer agreement affidavits are obtained from each patient prior to beginning the study.

Inclusion Criteria. The following patients are eligible for entry into the study:

1. Male or female patients > 18 yr and < 65 yr of age. Female patients are either previously surgically sterilized or postmenopausal (> 45 yr of age and no menstrual periods for at least 1 yr) or have a negative pregnancy test result prior to initiation into the study.

2. Patients admitted to the Institute within 48 h of burn injury.

3. Patients with burn wounds > 40% and < 75% of the total body surface area.

Exclusion Criteria. The following patients are excluded from the study:

1. Patients < 18 yr or > 65 yr of age.

2. Patients who are pregnant or nursing.

3. Patients with burns < 40% or > 75% of the total body surface area.

Case Report. On 8 February 1988, the initial patient, a 29-yr-old male with a 69.5% total body surface area burn, was enrolled in the study. A skin donor site on the left lateral calf measuring 58.5 cm^2 in size was harvested using the Padgett electric dermatome set at 1/10,000-in thickness. The harvested skin was then emersed in "Solution A" (4) in a 250-ml Erlenmeyer flask. The flask was sealed and placed on ice in a styrofoam transport box. It was then shipped to the Mayo Clinic overnight where it was received the next day and the harvested skin was started in the tissue culture protocol (4). Briefly, the skin was initially cut into 1-2 cm^2 pieces and treated with 0.17% trypsin for 30-45 min at 37°C. This treatment separates the epidermis from the dermis and the individual epidermal cells are dispersed and then collected. They are then entered into Phase I keratinocyte growth during which time MCDB-153 culture medium is employed. They were continued in Phase I for 2 wk at which point the quantity of keratinocytes produced was sufficient to allow initiation of the formation of cohesive multilayered sheets of keratinocytes. Approximately one-half of the cells produced at that point were frozen for future use and the remaining cells were changed to Phase II growth. In Phase II, the medium was switched to Dulbecco's modified Eagle medium containing 10% fetal bovine

serum and 1.8 mM calcium. Exactly 14 days after the initial harvest, the cells were converted to Phase II growth and 14 days later, 50 75-cm^2 flasks filled with confluent, multilayered keratinocytes sheets were shipped from the Mayo Clin to the Institute where they were placed in an incubator overnight. The following morning, the patient was taken to the operating room and under general anesthesia underwent excision and grafting with the cultured keratinocyte grafts. The area excised included unhealed regions on the chest, right flank, and right hip. The recipient wound bed was mainly a fresh deep dermal bed with a few areas of full-thickness skin loss scattered on the chest. In these areas of full-thickness skin loss, the grafts were applied to viable fat. There was no gross evidence of infection. The 50 sheets of cultured epithelium were then gently applied to these excised wounds, taking care to maintain proper orientation of the basal cell layer. Each piece measured approximately 6 X 5 cm once released from the tissue culture flask. Therefore, the area covered was approximately 1,600 cm^2. The cultured keratinocyte grafts were then covered with a silver-impregnated fine-mesh gauze which was moistened with 0.9% saline solution while in the operating room and covered with coarse-mesh pads stapled in place. Postoperatively, these dressings were moistened 3X daily using 5% mafenide acetate solution. During the same procedure, other burned areas on the patient's left arm and left foot were also excised and grafted with autograft meshed in a 1.5:1 fashion.

RESULTS

Seven days after excision and grafting, the initial dressing was removed. At that time, the cultured keratinocyte grafts were entirely nonviable. The grafts which had been transparent and "feathery" in appearance at the time of application now appeared to be approximately 1-mm thick and opaque. They were debrided off the wound with no evidence of adherence to the wound beds. Cultures obtained of these grafts showed no evidence of microorganisms. Histologic examination revealed that the keratinocytes were present but were necrotic. Bacteria or fungi were not seen in the tissue. The areas grafted during the same procedure with autograft experienced > 95% graft take.

DISCUSSION

The result in the case of this initial patient is understandably disappointing. The initial harvest of donor skin consisted of 58.5 cm^2 of skin. The donor site healed without problem and the tissue culture facility at the Mayo Clinic successfully produced 50 flasks of multilayered confluent keratinocyte grafts. The area eventually covered with these 50 flasks measured 1,600 cm^2. This amount of coverage from an initial donor site measuring only 58.5 cm^2

amounts to a 27-fold expansion ratio. This is by far a much larger expansion ratio than any other technique currently available can produce. However, the graft take on this initial patient was miserable. The wounds grafted with the cultured keratinocyte grafts all failed to heal and required regrafting with traditional meshed autograft at a later time. Possible explanations for this failure of graft take include infection, nonviability of the grafts following transportation, or destruction of the grafts secondary to either the silver-impregnated fine-mesh gauze or the 5% mafenide acetate solution to which they were exposed after application to the wound bed. Since cultures of the failed keratinocyte grafts and the wound bed itself demonstrated the absence of microorganisms, the likelihood that the graft failed due to infection is small. When initially removed from the tissue culture flasks, the grafts appeared quite viable; however, it is impossible to be sure that they were not injured during the 24-h transport and incubation period prior to application to the wounds. At this point, it is also impossible to be sure that they did not sustain an injury from their post-application treatment with either the silver-impregnated gauze or the 5% mafenide acetate solution. These cells are quite sensitive and fragile. The 5% mafenide acetate solution is the most likely noxious component to which the cultured grafts were exposed. Further studies on additional patients will have to be undertaken to determine if removing all 5% mafenide acetate solution from the cultured keratinocytes' environment will result in successful graft take.

In addition to determining whether or not the 5% mafenide acetate solution should be avoided, the studies should be continued to determine the optimal wound bed, e.g., fresh excision, granulation tissue, fascia, etc., for successful graft take and long-term durability of these grafts once they have successfully vascularized.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA312336	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	K	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	083
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) The Effect of High Frequency Ventilation on VA/Q Ratio in Sheep with Inhalation Injury

12. SUBJECT AREAS
06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 01	88 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.2	5
89	0.0	0

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Inhalation Injury; (U) High Frequency Ventilation; (U) Ventilation-Perfusion Ratio; (U) Cardiac Output; (U) Lab

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Animals: (U) Sheep; (U) RAII

23. (U) To compare the effects of volumetric diffusive ventilation and conventional ventilation on pulmonary and hemodynamic indices which are altered in an ovine inhalation injury model. A literature search was performed and indicated no duplication of effort.

24. (U) Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this Institute. Animals will be randomized to treatment with either conventional or high frequency ventilation. Changes in VA/Q as well as other pulmonary and physiologic measurements will be compared between groups.

25. (U) 8710 - 8809. This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 14 January 1987. Due to technical problems with the newly acquired mass spectrometer, the multiple inert gas elimination technique has not been operational. When the technique has been revalidated, the study will continue. This study was transferred to DA315355.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. * USGPO 1986-491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of High Frequency Ventilation on VA/Q in Sheep with Inhalation Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Takeshi Shimazu, MD
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of High Frequency Ventilation on VA/Q in Sheep with Inhalation Injury

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Takeshi Shimazu, MD
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

Severe inhalation injury has been shown to cause hypoxia, hypercarbia, and a shift of VA/Q to the left, i.e., increase in segments with VA/Q > 0 but < 1. Attempts to alter these derangements with conventional ventilation utilizing PEEP resulted in an increased dead space ventilation but had no significant effect on shunt or low VA/Q compartments. This study was designed to investigate the effects of high frequency percussive ventilation on these chnages.

Initial studies on 2 sheep, utilizing a moderate to severe inhalation injury, revealed the inability of high frequency percussive ventilation to oxygenate these animals as compared to conventional ventilation. This was believed to be secondary to the severe injury, with near total occlusion of the tracheobronchial tree with cast formation. Three subsequent sheep were studied using a mild to severe injury. Adequate ventilation and oxygenation was achieved with high frequency percussive ventilation as compared to conventional ventilation.

THE EFFECT OF HIGH FREQUENCY VENTILATION ON VA/Q IN SHEEP WITH INHALATION INJURY

The effect of inhalation injury on VA/Q utilizing the multiple inert gas elimination technique (MIGET) and cardiopulmonary parameters has been well described in an ovine model (1). Moderate to severe injury causes hypoxia, hypercarbia, and a shift of VA/Q to the left, i.e., increase in segments with VA/Q greater than zero but less than one. In addition, smoke-exposed animals show increased perfusion to shunt and low VA/Q segments. Attempts to alter these derangements with conventional ventilation utilizing positive end-expiratory pressure (PEEP) resulted in an increased dead space ventilation but had no significant effect on shunt or low VA/Q compartments (2).

High freqency ventilation has been proposed as a means of increasing ventilation to low VA/Q compartments. In a dog model using methacholine hydrochloride to induce low VA/Q compartments, Wagner was unable to demonstrate a beneficial effect of high frequency oscillation ventilation (3). This type of ventilator is relatively inefficient in terms of gas exchange and does not allow adequate ventilation of adult humans. Becuase of this difficulty and the inability of jet ventilators to adequately clear carbon dioxide, a hybrid type of ventilator has been developed that effects what is termed "volumetric-diffusive ventilation." This type of ventilator superimposes high frequency subtidal volume breaths on conventional convective breaths. In addition, PEEP is employed in an oscillatory nature. This ventilator is actually a flow interrupter and there is no active expiratory phase as seen in oscillation ventilation. Limited clinical use of this ventilator has demonstrated no adverse effects on cardiac parameters (4). In addition, salvage studies performed on patients with adult respiratory distress syndrome have suggested that previously unsalvageable patients have had reversal of their pulmonary process. The effect of this type of ventilation on disease processes which result in an increase in the number of low VA/Q compartments is unknown.

The purpose of this study is to compare volumetric diffusive ventilation with conventional ventilation in effecting changes in the pulmonary and hemodynamic parameters which are altered in an ovine inhalation injury model. If volumetric-diffusive ventilation can favorably affect VA/Q on inhalation injury, its application to humans with inhalation injury will be advantageous.

MATERIALS AND METHODS

Neutered male sheep weighing 25-45 kg will be utilized. Each sheep will be housed in a conventional outdoor run and

will have access to commercial feed and water ad libitum. Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this Institute (1).

Animals will be studied 24 h following smoke inhalation. On the day of the study, a peripheral venous catheter, a central venous pressure catheter, a ballon-directed thermodilution pulmonary artery catheter (7F, American Edwards Company, Irvine, CA), a lung water catheter (American Edwards Company), a femoral artery catheter, and an esophageal ballon will be inserted following induction of general anesthesia with alpha-chloralose (0.05 g/kg) and intubation. Animals will be paralyzed with pancronium bromide (0.03 to 0.04 mg/kg, Pavulon[R], Organon Pharmaceuticals, West Orange, NJ). After placement of all catheters, animals will be positioned prone and conventional mechanical ventilation will be continued with a volume-limited ventilator (Bear II[TM], Bear Medical Systems, Inc., Riverside, CA). Ventilator settings will be altered to maintain a pH between 7.35 and 7.40 and PO_2 between 80 and 100 mmHg. Lactated Ringer's will be constantly infused at a rate of 1 ml/kg/h. Central venous pressure (CVP) and pulmonary artery pressure (PAP) will be monitored with Statham P23Db transducers (Statham Instruments, Oxnard, CA) and systemic artery pressures with a Hewlett-Packard 1290A quartz transducer (Hewlett-Packard Company, Waltham, Massachusetts). Transpulmonary pressure will be monitored by a differential transducer (MP-451, Valadine Engineering Corporation, Northridge, CA). Inspiratory and expiratory gas concentration (N, O_2, and CO_2) will be monitored by a medical gas analyzer (MGA-1100, Perkin Elmer). Percutaneous O_2 saturation and PO_2 will be continuously monitored.

Heart rate, blood pressure, CVP, PAP, cardiac output, arterial blood gases, tidal volume, flow rates, transpulmonary pressures, and O_2 saturation will be measured every 30 min. Once the ventilator settings are maximized yielding a PO_2 between 80 and 100 mmHg and a pH between 7.35 and 7.40, the animal will be allowed to stabilize for 2 h. VA/Q distributions will then be measured utilizing the multiple inert gas elimination technique (MIGET). After stabilization, the lactated Ringer's infusion will be replaced with a lactated Ringer's solution containing 6 inert gases (sulphur hexafluoride, krypton, cyclopropane, halothane, ether, and acetone) which will be infused at a rate of 0.1 ml/kg/min. After 30 min, arterial and mixed venous blood will be drawn anaerobically into preweighed heparinized syringes (30 ml, matched, glass) simultaneously. Mixed-expired gas will be obtained from a temperature-controlled copper coil (OD = 3.49 cm, L = 640 cm) 1 min after obtaining the blood samples. Blood and expired gas samples will be analyzed immediately by a GC-MS (Model 5985, Hewlett-Packard). Repeat cardiopulmonary parameters will be measured at this time. MIGET data will be

stored and quantified by a software program on the Hewlett-Packard 1000 computer system.

The animals will then be disconnected from the conventional ventilator and switched to a volumetric-diffusive ventilator. Ventilator settings will be made to maintain a pH between 7.35 and 7.40 and a PO_2 between 80 and 100 mmHg. Cardiopulmonary parameters will then be measured every 30 min. The lactated Ringer's infusion containing the inert gases will be discontinued and lactated Ringer's at 1 cc/kg/h will be infused. After a 2-h stabilization period, VA/Q distribution will again be measured utilizing the MIGET. The animals will then be sacrificed.

Necropsies will be performed to document the extent of inhalation injury. A complete set of tissues will be fixed in 10% neutral buffered formalin and processed by standard methods. The locations of tissue sample collection sites will be midtrachea, tracheal bifucation, right and left proximal and distal bronchi, apical and diaphragmatic lobes, and any other morphologically significant foci.

Data following the stabilization period will be compared utilizing the student's t-test.

RESULTS

Two sheep with a 9-U smoke injury were studied. Due to technical problems with the MIGET, no VA/Q data from these sheep are available. High frequency ventilation failed to adequately oxygenate these animals as compared to conventional ventilation.

Three additional sheep with a 6-U smoke injury were also studied. There was no difference between high frequency ventilation and conventional ventilation in maintenance of normocapnia or ability to oxygenate these animals.

DISCUSSION

Upon resolution of the technical problems with the MIGET, further progress on this study should be made.

PRESENTATIONS/PUBLICATIONS

None.

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3. Kaisor HG, Davies NJH, Rodriguez RR, et al: Efficacy of high-frequency ventilation in presence of extensive ventilation-perfusion mismatch. **J Appl Physiol** 58:996-1004, 1985.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA312334	88 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
87 10 01	K	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	085
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

12. SUBJECT AREAS
06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 01	88 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Basil A. Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	0.5	35
89	0.0	0

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available): MASON, A D
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available): OKERBERG, C V

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Shock; (U) Resuscitation; (U) Hemodynamics; (U) Plasmaphoresis; (U) Fluid Replacement; (U) Albumin;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Crystalloids; (U) Lab Animals: (U) Sheep; (U) ILIR; (U) RAII

23. (U) To determine the hemodynamic consequences of controlled pure plasma loss in sheep using a method to simulate the acute burn. Subsequently, response to therapy will be assessed in burned soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) A plasmaphoresis filter will be used to produce intravascular plasma loss similar to that caused by burn injury. This device selectively removes plasma while leaving the formed elements of blood in the vascular system.

25. (U) 8710 - 8809. Eight animals were studied during this reporting period and the model has been established in a reliable fashion. Animals subjected to pure plasma loss have been resuscitated with several resuscitation schema, including crystalloid and colloid fluids. Data from these 8 animals are currently being analyzed. An addendum is being written which will validate the model by comparing the hemodynamic changes seen in a 50% burn model with those seen in the pure plasma volume loss model. This project was transferred to DA315356.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

1 October 1987 - 30 September 1988

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Carlin V. Okerberg, DVM, PhD, Major, VC
Brian C. Rakestraw, SGT
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012

PERIOD COVERED IN THIS REPORT: 1 Oct 87 through 30 Sep 88

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Carlin V. Okerberg, DVM, PhD, Major, MC
Brian C. Rakestraw, SGT
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

A controlled plasma loss ovine model designed to simulate the rate of intravascular plasma loss in the acute burn has been developed. Fluid replacement therapy will be correlated with the amount of plasma loss while measuring hemodynamic changes in this animal model. Preliminary studies have been completed which have demonstrated the necessity for splenectomy in this model. This standardized model will now be used to evaluate the effects of varying resuscitation formulae on a pure plasma loss model. In the near future, this model will be compared to pure plasma loss seen in an ovine model of thermal injury.

EFFECTS OF REPLACEMENT THERAPY ON HEMODYNAMIC PARAMETERS IN AN OVINE MODEL OF CONTROLLED PURE PLASMA LOSS

Many models have been employed to explore the physiologic and pathophysiologic sequelae of shock, but most have dealt with loss of formed blood elements along with plasma loss. The question which remains to be answered is to what extent the hemodynamic response in the shock model relates to the loss of plasma per se without red blood cell loss. A controlled plasma loss designed to simulate the rate of intravascular plasma loss in the acute burn period has been developed by the interposition of a plasmaphoresis filter between the arterial and venous circulation of experimental animals. This design will allow the simulation of plasma loss of the acute burn which accounts for hemodynamic instability (1-2). In previous work (3), this model has shown efficacy as a pure plasma loss shock model, albeit an accelerated representation of the burn state. With the control of plasma flux to more closely represent burn shock in a temporal sense, hemodynamic changes can be better defined. Subsequent fluid replacement therapy can then be effected to form the scientific basis for postburn resusitation in humans.

MATERIALS AND METHODS

The effects of intravascular loss of plasma on cardiovascular performance will be investigated in 20 one- to two-year-old, random source, nonpregnant female sheep weighing 24-40 kg. During the first stage of the study, the animals are prepared under general anesthesia by cannulation of the right femoral artery for blood sampling, the right jugular vein for hemodynamic monitoring, and the left jugular vein and left carotid artery for ultrafiltration. Aortic, central venous, pulmonary artery, left atrial, and pulmonary capillary wedge pressure are recorded (Hewlett-Packard Model 7754A) using calibrated pressure transducers (Hewlett-Packard Model 1290A). Arterial blood gas and cardiac output by the thermodilution method (Edwards Model 9520) are also determined. A Foley catheter is introduced for urine output monitoring. The animals are placed in metabolic cages for 2 days and fed *ad libitum* while recovering from the initial procedure. During the second stage of the study, the animals are heparinized and plasmaphoresis is initiated using an Asahi Plasma Separator (Parker Hannifin Corporation) after baseline measurements of cardiovascular and respiratory indexes and sampling of blood for electrolyte, blood gas, and coagulation determinations. This system has a cellulose acetate hollow fiber core which allows for passage of plasma, but not cellular elements. The unanesthetized animals are subjected to a selective plasma extraction (Fig 1) at a plasma flux designed to simulate the rate of loss in the acute burn period as described by Pruitt *et al* (4).

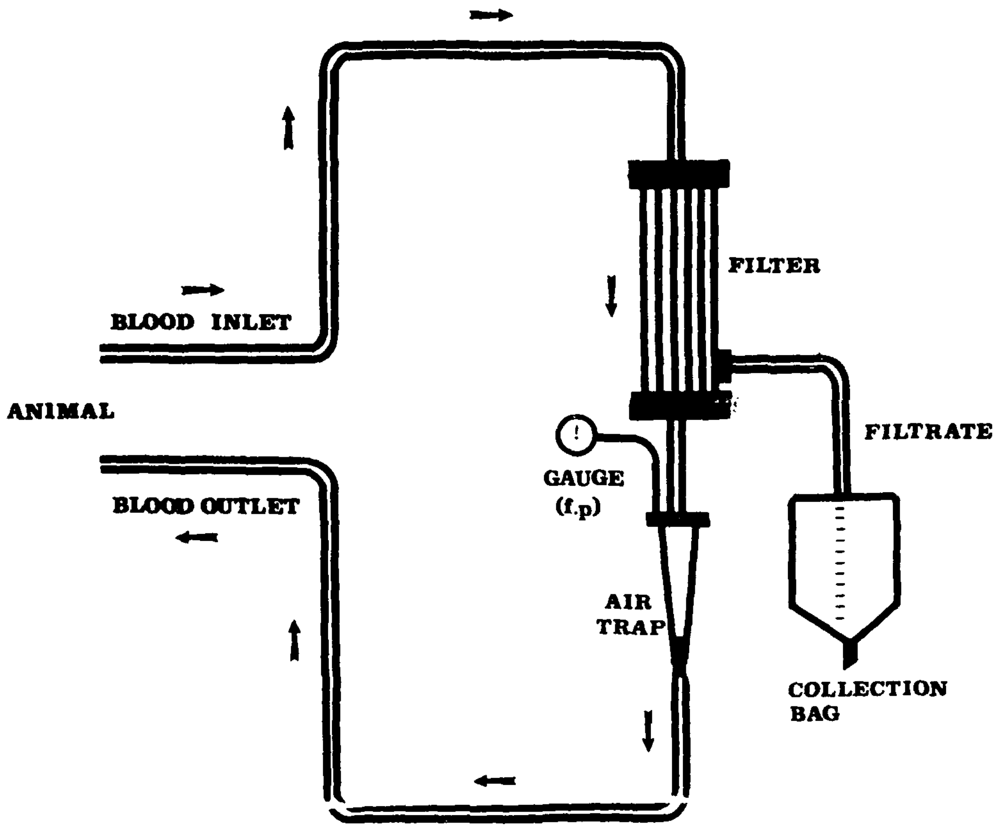

FIGURE 1. Graphic representation of the filtration circuit.

RESULTS

Eight animals were studied during this reporting period and the model has been established in a reliable fashion, with the need for prior splenectomy recognized. Animals subjected to pure plasma loss have been resuscitated with several resuscitation schema, including crystalloid and colloid fluids.

DISCUSSION

Data from these 8 animals is currently being analyzed. A 50% total body surface area burn has been chosen as the initial model for study. Plasma loss will be adjusted on an hourly basis to meet these losses. Ongoing measurements of hemodynamic parameters will be conducted, to include systolic blood pressure, left atrial pressure, pulmonary capillary wedge pressure, cardiac output, hematocrit, serum chemistries (electrolytes, blood urea nitrogen, creatinine, glucose), serum osmolality, and urine output. After 2 h of plasma loss, fluid resuscitation will begin utilizing several of the most popular burn resuscitation formulae. One group will be resuscitated

via the modified Brooke formula, another using the Parkland formula, and another using hypertonic saline. Initial plasma volume will be measured utilizing Evans' blue prior to institution of plasma loss. All animals will be fully heparinized prior to institution of plasmaphoresis. An addendum is being written which validate the model by comparing the hemodynamic changes seen in a 50% burn model with those seen in the pure plasma volume loss model.

PRESENTATIONS/PUBLICATIONS

None.

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US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

PRESENTATIONS

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Carlson DE: Combat field feeding system force development test and experimentation. Presented to Dietetic Interns, Nutrition Care Division, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 8 October 1987.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 15 October 1987.

Burleson DG: Measurement of peripheral blood lymphocyte subpopulations in burned patients. Presented at the 11th International RES Congress and 24th National Reticuloendothelial Society, Kauai, Hawaii, 20 October 1987.

Hollan E: Infection control in the AIDS era. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 21 October 1987.

Pruitt BA Jr: Overview of the activities of the US Army Institute of Surgical Research. Presented at the BioVision '87 Conference, San Antonio, Texas, 22 October 1987.

Vaughan GM: Control of TSH after burn injury. Presented at the 4th Annual Meeting of the US Army Regional Meeting of the American College of Physicians, San Francisco, California, 25 October 1987.

Chu C-S: Effects of low voltage direct current on the burn wound. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

McManus WF: Management of patients with massive burns. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

Pruitt BA Jr: Four decades of progress in burn care and research. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

Shimazu T: Effects of smoke inhalation injury on ventilation-perfusion ratio of the lung. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

Burleson DG: Effect of burn injury and infection on lymphocyte populations. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 26 October 1987.

Kim SH: Histologic classification of burn wound infections. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 October 1987.

McManus AT: The rise and fall of Pseudomonas. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 October 1987.

Vaughan GM: Central nervous system alterations in flow-phase burn injury. Presented at the 40th Anniversary Symposium at the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 October 1987.

Gutierrez RT: PT/OT in the management of burns. Presented to Physical Therapy Assistant, Occupational Therapy, and Licensed Vocational Nurse students at St. Phillips College, San Antonio, Texas, 28 October 1987.

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Luster SH: Occupational therapy rehabilitation in burn care. Presented to Physical Therapy Assistant, Occupational Therapy, and Licensed Vocational Nurse students, St. Phillips College, San Antonio, Texas, 29 October 1987.

Zelenka JP: New strategies in occupational therapy in burn care. Presented at the Annual Great Southern Occupational Therapy Conference, New Orleans, Louisiana, 29 October 1987.

Kim SH: Clinical sepsis and burn wound biopsy. Presented at the 81st Annual Scientific Assembly of the Southern Medical Association, San Antonio, Texas, 1 November 1987.

Pruitt BA Jr: Current techniques of burn wound care. Presented at the 81st Annual Scientific Assembly of the Southern Medical Association, San Antonio, Texas, 2 November 1987.

Duncan DJ: Pediatric burn patients: Are they different? Presented to the Northeast Regional Recruiting Command, St. Mary's College, Newburgh, New York, 2-3 November 1987.

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Pruitt BA Jr: Effect of injury on host defenses. Presented at the Department of Surgery Trauma Meeting, Wayne State University, Detroit, Michigan, 5-7 November 1987.

Pruitt BA Jr: Nonbacterial infections in burn patients. Presented at the Department of Surgery Trauma Meeting, Wayne State University, Detroit, Michigan, 5-7 November 1987.

Keenan JR: Initial management of the burn patient. Presented at St. Phillips College, San Antonio, Texas, 10 November 1987.

McManus WF: Burn disaster management. Presented at the 94th Annual Meeting of the Association of Military Surgeons of the United States, Las Vegas, Nevada, 10 November 1987.

Pruitt BA Jr: Burn wound care and treatment for chemical burns, burn patient transfer and transport. Presented to the Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 10 November 1987.

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Gutierrez RT: Physical therapy in natural disasters. Presented at the 94th Annual Meeting of the Association of Military Surgeons of the United States, Las Vegas, Nevada, 10 November 1987.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 19 November 1987.

Hollan E: Infection control in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 1 December 1987.

Gutierrez RT: Physical therapy in burn care. Presented to the Physical Therapy Advanced Course, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 1 December 1987.

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McManus WF: Treatment of special burns: Chemical, bitumen, and electric. Presented at the Annual Meeting of the American Academy of Dermatology, San Antonio, Texas, 5 December 1987.

Latona PS: Initial management of the burn patient. Presented at the Brooks Aerospace School of Medicine, Brooks Air Force Base, San Antonio, Texas, 9 December 1987.

Latona PS: Initial management of the burn patient. Presented to the Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 10 December 1987.

Keenan JR: Initial management of the burn patient. Presented to the Intensive Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 10 December 1987.

Pruitt BA Jr: Etiology and pathogenesis of inhalation injury. Presented at the Burn Seminar, International Society for Burn Injuries, Denver, Colorado, 10 December 1987.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 17 December 1987.

Carlson DE: Nutritional care of the critically ill. Presented to the Brooke Army Medical Center Dietetic Interns, Fort Sam Houston, San Antonio, Texas, 15 January 1988.

Zelenka JP: Occupational therapy in burn care. Presented to the Occupational Therapy Course (91L), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 20 January 1988.

Waymack JP: The effect of blood transfusions on resistance to infectious complications. Presented at the Symposium on Biologic Effect of Blood Transfusions on Immune Function, Snowbird, Utah, 21 January 1988.

Duncan DJ: Standards of nursing care for the large burn patient in the initial 48 hours. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Hollan E: The importance of infection control. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Jordan BS: Management of pain. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

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Pruitt BA Jr: Burn injury as a military problem: epidemiology and triage. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Summers TM: Psychosocial complications of burn injuries. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in surgical patients. Presented to the Cincinnati Surgical Society, Cincinnati, Ohio, 2-3 February 1988.

Pruitt BA Jr: Current therapy of burns. Presented to the Officer Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1988.

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Gutierrez RT: Thermal injuries: Physical therapy management. Presented to the Physical Therapy Specialists Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 10 February 1988.

Summers TM: Families in crisis. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 February 1988.

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Luster SH: Rehabilitation procedures in burn care. Presented to Occupational and Physical Therapy Section, St. Rose Rehabilitation Center, San Antonio, Texas, 16 February 1988.

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Luster SH: Rehabilitation of acute burn patients. Presented to the Occupational and Physical Therapy Section, St. Rose Rehabilitation Center, San Antonio, Texas, 17 February 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 18 February 1988.

Pruitt BA Jr: Opportunistic infections in severely injured patients. Presented to the Department of Surgery, University of Texas Health Science Center, Dallas, Texas, 20 February 1988.

Pruitt BA Jr: Current concepts of burn therapy. Presented at the Quarterly Trauma Conference, Uniformed Services University of the Health Sciences, Bethesda, Maryland, 25-26 February 1988.

Pruitt BA Jr: The diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, Bethesda, Maryland, 26 February 1988.

Pruitt BA Jr: Current treatment of the burn wound. Presented at the Department of Surgery Grand Rounds, University of Nebraska Medical Center, Omaha, Nebraska, 26-27 February 1988.

Latona PS: Initial management of the burn patient. Presented to the Combat Medical Specialty Division, Fort Sam Houston, San Antonio, Texas, 4 March 1988.

Keenan JR: Initial management of burn patients. Presented to the Intensive Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 10 March 1988.

Waymack JP: The effect of blood transfusions on immune function. Presented at the First International Congress on the Immune Consequences of Trauma Shock and Sepsis Symposium, Munich, Germany, 10 March 1988.

Pruitt BA Jr: Presentation as National Faculty Member to Advanced Burn Life Support Course. Presented to the Department of Surgery, University of North Carolina School of Medicine, Chapel Hill, North Carolina, 10-11 March 1988.

Pruitt BA Jr: Fluid resuscitation of the injured patient. Presented to the 1988 UCLA Extension Course, Palm Springs, California, 13-17 March 1988.

Pruitt BA Jr: Management of the complications of IV therapy. Presented to the 1988 UCLA Extension Course, Palm Springs, California, 13-17 March 1988.

Cioffi WG Jr: Early care and aeromedical transport of the thermally injured patient. Presented at the National Aerospace Conference, New Orleans, Louisiana, 16 March 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 17 March 1988.

Cioffi WG Jr: Electrical injury. Presented at the 42nd Annual Texas Job Training and Safety Conference, Corpus Christi, Texas, 17 March 1988.

Vaughan GM: Hypodipsia-hypernatremia. Presented at the Endocrine Combined Conference, University of Texas Health Science Cener, San Antonio, Texas, 17 March 1988.

Summers TM: Psychosocial aspects of thermal injuries. Presented at the Tri-Service Reserve Burn Nursing Seminar, Portland, Oregon, 19 March 1988.

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Luster SH: Rehabilitation procedures in burn care. Presented at the 45th Station Hospital, Vancouver, Washington, 20 March 1988.

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Gutierrez RT: Physical therapy in burn care. Presented to the Advanced Physical Therapy Course, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 24 March 1988.

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McManus WF: Surgical nutrition - indications and problems. Presented at the Gary P. Wratten Surgical Symposium, Bethesda, Maryland, 30 March 1988.

Summers TM: USAISR Nursing Service. Presented to the Educators Tour, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 April 1988.

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Pruitt BA Jr: Evolution and revolution in the management of the seriously burned. Presented as the Excelsior Surgical Society/Edward D. Churchill Lecturer at the 16th Annual Meeting of the American College of Surgeons, San Antonio, Texas, 11 April 1988.

Duncan DJ: Army practical nurses: Are they prepared to perform as head nurses and practical nursing instructors expect them to perform? Presented at the Phyllis J. Verhonick Nursing Research Seminar, Washington, DC, 11 April 1988.

Pruitt BA Jr: Fluid resuscitation of injured patients. Presented to the Department of Surgery, Surgical Grand Rounds, New Jersey Medical School, Newark, New Jersey, 18 April 1988.

Pruitt BA Jr: Care of the burn wound. Presented at Hackensack Hospital, Hackensack, New Jersey, 19 April 1988.

Guiterrez RT: The role of physical therapists in a natural disaster. Presented at the Central District Meeting of the Texas American Physical Therapy Association, San Antonio, Texas, 19 April 1988.

Jordan BS: The posttrauma surgical reconstructive patient - the continuum of burn nursing care: Current concepts in burn nursing. Presented at the University of New York, Elmira, New York, 20 April 1988.

Molter NC: Standards of nursing care for the large burn patient in the initial hours. Presented at the University of New York, Brockport, New York, 20 April 1988.

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Jordan BS: The posttrauma surgical reconstructive patient - the continuum of burn nursing care: Current concepts in burn nursing - Presented at the American Association of Critical Care Nurses Annual Education Conference, Buffalo, New York, 21 April 1988.

Pruitt BA Jr: Current concepts in burn management. Presented at the Grand Rounds, Tulane University Medical Center, New Orleans, Louisiana, 23 April 1988.

Molter NC: Extended role of the nurse. Presented to the Military Postgraduate Critical Care Course, Washington, DC, 26 April 1988.

Duncan DJ: Initial management of burn victims. Presented to the Luling Emergency Medical Services, Luling, Texas, 28 April 1988.

Maly DW: Aeromedical transport of the burn victim. Presented to the Luling Emergency Medical Services, Luling, Texas, 28 April 1988.

Zelenka JP: Occupational therapy in burn care. Presented to Occupational Therapy Course (91L), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 2 May 1988.

Foresman JL: Thermal injuries - physical therapy management. Presented to the Physical Therapy Specialist Course Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 May 1988.

Latona PS: Initial management of the burn victim. Presented to the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 4 May 1988.

Burleson DG: The effect of intravenous immune globulin administration on lymphocyte phenotype and function in burnd patients. Presented at the 8th Annual Meeting of the Surgical Infection Society, San Francisco, California, 5 May 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in injured man. Presented as the Marion Donald Lecturer at the Annual Meeting of the Alabama Chapter of the American College of Physicians, Mobile, Alabama, 11-12 May 1988.

Pruitt BA Jr: Fluid resuscitation of surgical patients. Presented as the Marion Donald Lecturer at the Annual Meeting of the Alabama Chapter of the American College of Physicians, Mobile, Alabama, 11-12 May 1988.

Jordan BS: Wound management and complications of burn injury. Presented at Seton Medical Center, Austin, Texas, 13 May 1988.

Maly DW: Aeromedical transport of the burn victim. Presented at Seton Medical Center, Austin, Texas, 13 May 1988.

McManus WF: Prehospital care of the burn victim. Presented at Seaton Hospital, Austin, Texas, 13 May 1988.

Summers TM: Psychosocial nursing and the burn patient. Presented at Seton Medical Center, Austin, Texas, 13 May 1988.

Pruitt BA Jr: Epidemiology, triage, and aeromedical transfer of burn patients. Presented to the Combat Casualty Management Course (C4A), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 May 1988.

Pruitt BA Jr: Burn care as a military problem. Presente to the AMEDD Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 17 May 1988.

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Gutierrez RT: Physical therapy management of burn patients. Presented to the Physical Therapy Specialists Course

(91J), Academy of Health Sciences, Fort Sam Houston, Texas, 20 May 1988.

Luster SH: A checklist for identification of rehabilitation problems in burn patients. Presented at the Annual Meeting of the American Trauma Society, Arlington, Virginia, 20 May 1988.

Molter NC: Acute pain management: Clinical decision-making responsibilities for the professional nurse. Presented to the 2nd Aeromedical Evacuation Squadron, Rhein Main Air Force Base, Germany, 20 May 1988.

Carlson DE: Nutritional needs of the burn patient. Presented at the Patients' Family Group Meeting, US Army Institute of Surgical Research, Fort Sam Houston, Texas, 24 May 1988.

Duncan DJ: Initial management of burn victims. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Hollan E: Infection control procedures in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Jordan BS: General nursing care of the burn wound. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Jordan BS: Principles of hemodynamic monitoring in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

McDaniel J: Documentation in the intensive care unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Summers TM: Communicating effectively. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Summers TM: Psychosocial aspects of thermal injuries. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Wright M: Perioperative care of the burn patient. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Cioffi WG Jr: Aeromedical transport of the thermally injured patient. Presented to the Aerospace Medical Association, New Orleans, Louisiana, 1 June 1988.

Vaughan GM: Entrainment of both rise and fall of rat serum melatonin in cycling lighting. Presented at the 70th Annual Meeting of the Endocrine Society, New Orleans, Louisiana, 9 June 1988.

Gutierrez RT: Getting mobilized to El Salvador. Presented to the Physical Therapy Specialists Advanced Course (91J), Acadmey of Health Sciences, Fort Sam Houston, San Antonio, Texas, 13 June 1988.

Gutierrez RT: Physical therapy and thermal injuries. Presented to the Physical Therapy Specialists Advanced Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 13 June 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in critically ill patients. Presented at the 8th Annual Teaching Day, St. Francis Hospital and Medical Center, Hartford, Connecticut, 15 June 1988.

Pruitt BA Jr: Fluid resuscitation of injured patients. Presented at the 8th Annual Teaching Day, St. Francis Hospital and Medical Center, Hartford, Connecticut, 15 June 1988.

DePew CL: Fluid and electrolyte pathophysiology. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 15-16 June 1988.

Summers TM: Crisis and families. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 15-16 June 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 16 June 1988.

Gutierrez RT: The role of physical therapy in El Salvador. Presented to the United States Army Medical Specialist Corps, Fort Sam Houston, San Antonio, Texas, 17 June 1988.

DePew CL: Pacemakers. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 28 June 1988.

Summers TM: Stress and crisis management. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 1 July 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in burn patients. Presented as the Kirk Anderson Traber Memorial Lecturer, University of Texas Medical Branch and Shriners Burns Institute, Galveston, Texas, 6-7 July 1988.

Vaughan GM: The pineal and its relation to burn injury and human physiology. Presented to Anatomy Department, Mahidol University, Bankok, Thailand, 18 July 1988.

Duncan DJ: Initial management of the burn victim. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 July 1988.

Jordan BS: Standards of nursing care for the large burn victim in the initial 48 hours. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 July 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 21 July 1988.

Vaughan GM: Daytime unresponsiveness of the human and hamster pineal to adrenergic stimulation. Presented at the Satellite Symposium of the 8th International Congress of Endocrinology, Hong Kong, 27 July 1988.

Vaughan GM: Entrainment of rat serum melatonin (MEL) rise and fall in cyclic lighting. Presented at the Satellite Symposium of the 8th International Congress of Endocrinology, Hong Kong, 28 July 1988.

Vaughan GM: The endocrine response to burn injury. Presented to the Departments of Medicine and Pathophysiology, First Medical College of the Peoples' Republic of China, 29 July 1988.

Duncan DJ: Management of burn victims in the theater of operations. Presented at the 2nd Annual Joint Military Trauma Symposium, United States Air Force Academy, Colorado Springs, Colorado, 3 August 1988.

Selzer RA: Management of burn victims in the theater of operations. Presented to the United States Air Force

Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 3 August 1988.

Luster SH: Occupational therapy in burn care. Presented to the Occupational Therapy Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 August 1988.

Pruitt BA Jr: Current treatment of the burn wound. Presented to the Department of Surgery, Ben Taub Hospital, Houston, Texas, 6 August 1988.

Luster SH: A checklist for identification of rehabilitation problems in burn patients. Presented at the Annual Army Medical Specialist Corps Symposium, Leesburg, Virginia, 8 August 1988.

Vaughan GM: Pituitary. Presented to the Endocrine Pathophysiology Sophomores, Department of Medicine, University Texas Health Science Center, San Antonio, Texas, 8 August 1988.

Vaughan GM: Thyroid. Presented to the Endocrine Pathophysiology Sophomores, Department of Medicine, University Texas Health Science Center, San Antonio, Texas, 8 August 1988.

Beverly ED: Liver disease. Prexented to the Practical Nurse Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 August 1988.

Carlson DE: Nutritional needs of the burn patient. Presented at the Patients' Family Group Meeting, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 August 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 18 August 1988.

Pruitt BA Jr: Clinical and laboratory studies of acute inhalation injury. Presented at the Annual Meeting of the International Surgical Group, Reykjavik, Iceland, 21-22 August 1988.

Molter NC: USAISR Nursing Service. Presented to the Educators Tour, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 23 August 1988.

Pruitt BA Jr: Inhalation injury and airway management. Presented to the American Burn Life Support Instructor Course, Pittsburgh, Pennsylvania, 26-27 August 1988.

Pruitt BA Jr: Transfer and transport of burn patients. Presented to the American Burn Life Support Instructor Course, Pittsburgh, Pennsylvania, 26-27 August 1988.

Broberg R: Initial management of burn victims. Presented to the Combat Medical Specialty Division, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 1 September 1988.

McManus AT: Occurrence of Pseudomonas aeruginosa in seriously burned patients: A review of 950 patients (1983-1987). Presented at the International Symposium on Basic Research and Clinical Aspects of Pseudomonas aeruginosa infections, Copenhagen, Denmark, 1 September 1988.

Pruitt BA Jr: Burn wound excision and the null hypothesis. Presented at the Annual Meeting of the Halsted Society, Jackson Hole, Wyoming, 6-9 September 1988.

Keenan JR: Initial management of burn victims. Presented to the 32nd Aeromedical Evacuation Squadron, Kelly Air Force Base, San Antonio, Texas, 10 September 1988.

Dorsey CS: Role of the LVN on the burn flight team. Presented to the Practical Nurse Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 12 September 1988.

Dimmick DJ: Role of the LVN in the Clinical Data Division, US Army Institute of Surgical Research. Presented to the Practical Nurse Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 12 September 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, University of North Carolina, Chapel Hill, North Carolina, 12-13 September 1988.

Pruitt BA Jr: Fluid resuscitation of injured man. Presented to the Department of Surgery, University of North Carolina, Chapel Hill, North Carolina, 12-13 September 1988.

Duncan DJ: Initial management of the burn victim. Presented to the School of Nursing, Baptist Memorial Hospital, San Antonio, Texas, 13 September 1988.

Duncan DJ: Standards of nursing care for the large burn victim in the initial 48 hours. Presented to the School of Nursing, Baptist Memorial Hospital, San Antonio, Texas, 13 September 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 13 September 1988.

Anderson SE: Standards of nursing care for the large burn victim in the initial 48 hours. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

DePew CL: Documentation in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Duncan DJ: Initial management of the burn victim. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Hollan E: Infection control in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Jordan BS: General nursing care of the burn wound. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Keenan JR: Aeromedical transport of thermally injured patients. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

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